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(54) Title: G PROTEIN COUPLED RECEPTOR PROTEIN, PRODUCTION, AND USE THEREOF

(57) Abstract

DNA primers effective in screening G protein coupled receptor protein-encoding DNA fragments are provided. The primers which are complementary to nucleotide sequences that are in community with (homologous to) the nucleotide sequences encoding amino acid sequences corresponding to or near the first membrane-spanning domain or the sixth membrane-spanning domain each of known various G protein coupled receptor proteins were designed and synthesized. Methods of amplifying G protein coupled receptor protein-encoding DNAs using the above DNA primers, and novel target G protein coupled receptor protein-encoding DNAs are also provided. Screening of DNA libraries can be efficiently carried out. Human pituitary gland or amygdala-derived and mouse pancreas-derived G protein coupled receptor proteins, etc., or salts thereof, partial peptides thereof, DNAs coding for the above G protein coupled receptor proteins, processes for producing the above G protein coupled receptor proteins, methods of determining ligands for the above G protein coupled receptor proteins, methods of screening compounds that inhibit the binding between the ligand and the G protein coupled receptor proteins or screening kits therefor, compounds or salts thereof obtained by the above screening method or the screening kit, pharmaceutical compositions containing the above compounds or salts thereof, and antibodies against the above protein coupled receptor proteins or partial peptides thereof are provided.

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DESCRIPTION

G PROTEIN COUPLED RECEPTOR PROTEIN,
PRODUCTION, AND USE THEREOFFIELD OF THE INVENTION

5 The present invention relates to novel DNAs which are useful as DNA primers for a polymerase chain reaction (PCR); methods for amplifying DNAs each coding for a G protein coupled receptor protein via PCR techniques using said DNA; screening methods for DNAs each encoding a G protein coupled receptor protein via PCR techniques using said DNA; 10 G protein coupled receptor protein-encoding DNAs obtained by said screening method; G protein coupled receptor proteins which are encoded by the DNA obtained via said screening method, peptide fragments or segments thereof, and modified peptide derivatives thereof; etc.

15 The present invention also relates to novel G protein coupled receptor proteins; novel G protein coupled receptor protein-encoding DNAs; processes for producing said G protein coupled receptor protein; use of said receptor protein and said protein-encoding DNA; etc.

20 The present invention also relates to novel human amygdaloid nucleus-derived G protein coupled receptor proteins; novel DNAs each coding for said G protein coupled receptor protein; processes for producing said G protein coupled receptor protein; use of said receptor protein and said protein-encoding DNA; etc.

25 The present invention also relates to novel mouse pancreatic β cell line MIN6-derived G protein coupled receptor proteins; novel DNAs each coding for said G protein coupled receptor protein; processes for producing said G protein coupled receptor protein; use of said receptor protein and

said protein-encoding DNA; etc. Further, the present invention relates to novel human-derived G protein coupled receptor proteins (human prinoceptors); novel DNAs each coding for said G protein coupled receptor protein; processes for producing 5 said G protein coupled receptor protein; use of said receptor protein and said protein-encoding DNA; etc.

BACKGROUND OF THE INVENTION

A variety of hormones, neurotransmitters and the like 10 control, regulate or adjust the functions of living bodies via specific receptors located in cell membranes. Many of these receptors mediate the transmission of intracellular signals via activation of guanine nucleotide-binding proteins (hereinafter, sometimes referred to as G proteins) with which the receptor is coupled and possess the common (homologous) structure, i.e. 15 seven transmembranes (membrane-spanning regions (domains)). Therefore, such receptors are generically referred to as G protein coupled receptors or seven transmembrane (membrane-spanning) receptors.

G protein coupled receptor proteins have a very 20 important role as targets for molecules such as hormones, neurotransmitters and physiologically active substances, which molecules control, regulate or adjust the functions of living bodies. Each molecule has its own receptor protein which is specific thereto, whereby the specificities of individual 25 physiologically active substances, including specific target cells and organs, specific pharmacological actions, specific action strength, action time, etc., are decided. Accordingly, it has been believed that, if G protein coupled receptor genes or cDNA can be cloned, those will be helpful not only for the 30 clarification of structure, function, physiological action, etc. of the G protein coupled receptor but also for the development of pharmaceuticals by investigating the substances which act on the receptor. Until now, only several G protein coupled receptor genes or cDNAs have been cloned but it is 35 believed that there are many unknown G protein coupled receptor genes which have not been recognized yet.

The characteristic feature of the G protein coupled receptor proteins which have been known up to now is that seven clusters of hydrophobic amino acid residues are located in the primary structure and pass through (span) the cell membrane at each region thereof. It has been known that such a structure is common among all of the known G protein coupled receptor proteins and further that the amino acid sequences corresponding to the area where the protein passes through the membrane (membrane-spanning region or transmembrane region) and the amino acid sequences near the membrane-spanning region are often highly conserved among the receptors.

When an unknown protein has such a structure, it is strongly suggested that said protein is within a category of the G protein coupled receptor proteins. In addition, some amino acid residue alignments are common (homologous) and, by taking it as a characteristic feature, it is further strongly suggested that said protein is a G protein coupled receptor protein.

Libert, F., et al. (Science, 244:569-571; 1989) reported a method for cloning novel receptor genes by means of a polymerase chain reaction (hereinafter, sometimes referred to as PCR or a PCR technique) for a synthetic DNA primer which was synthesized based upon the information of common amino acid sequences obtained from a comparison among known G protein coupled receptor proteins. Libert, F. et al. used a pair of synthetic DNA primers corresponding to the portions of the third and the sixth membrane-spanning regions. However, in general, the design of primers used for the PCR regulates the molecular species of DNAs which are to be amplified.

In addition, when a similarity (homology) in the amino acid sequence level is used as a basis, the use of different codons affects on the binding (hybridization) of the primer thereby resulting in a decrease in the amplifying efficiency.

Accordingly, although various novel receptor protein DNAs have been obtained using said DNA primers, it is not possible to succeed in amplifying DNAs for all receptor proteins in the prior art.

Further, the amino acid sequence which is common to from the first to the seventh membrane-spanning regions among 74 G protein coupled receptor proteins was reported by William C. Probst, et al. (DNA and Cell Biology, Vol. 11, No. 1, 5 1992, pp. 1-20). In this report, however, there is no suggestion for a method in which DNA coding for a novel G protein coupled receptor protein is screened by means of PCR using DNA primers which are complementary to the DNA coding for those amino acid sequences.

10 It would be desirable to develop DNA primers for PCR techniques which allow selective and efficient screenings of DNAs coding for the areas (regions) more nearer the full length of novel G protein coupled receptor proteins by utilizing the common (homologous) sequence(s) of the G protein coupled 15 receptor protein or the DNA coding therefor.

It would also be desirable to develop synthetic DNA primers corresponding to the portions of the third and the sixth membrane-spanning regions, said primer being useful in screening for DNA coding for G protein coupled receptor 20 proteins in more selective and efficient manner as compared with a series of the synthetic DNA primers corresponding to the sequences of the third to the sixth membrane-spanning regions as reported by Libert, F. et al.

G protein coupled receptor proteins are important for 25 investigating substances which control the function of living organisms and proceeding developments thereof as pharmaceuticals. Finding and development of candidate compounds for new pharmaceuticals can be efficiently proceeded by using G protein coupled receptor proteins and by conducting 30 receptor binding experiments and evaluating experiments on agonists/antagonists using intracellular information transmittance systems as indexes. Especially when the presence of a novel G protein coupled receptor protein can be clarified, the presence of a substance having a specific action thereon 35 can be suggested.

If a novel DNA which codes for a novel G protein coupled receptor protein can be efficiently screened and

isolated, it will now be possible to proceed with the isolation of DNA having an entire coding region, the construction of an expression system therefor and the screening of an acting ligand.

5 A hypothalamo-hypophysial system is one of the passages for controlling, regulating or adjusting the functions of organisms relying upon interactions of hormones and neurotransmitters with G protein coupled receptors. In the hypothalamo-hypophysial system, the secretion of pituitary 10 hormones from the pituitary body (hypophysis) is regulated by hypothalamic hormones (hypophysiotropic releasing factors), and the functions of target cells and organs are controlled by pituitary hormones released into the blood. Functions which are important for the living body are regulated through this 15 system, such as maintenance of homeostasis and control of development and growth of a genital system and an individual organism. Representative examples of the hypothalamic hormones include TRH, LH-RH, CRF, GRF, somatostatin, galanin, etc. Representative examples of the pituitary hormones include TSH, 20 ACTH, FSH, LH, prolactin, growth hormone, oxytocin, vasopressin, etc. In particular, the secretion of pituitary hormones is regulated according to a positive feedback mechanism or a negative feedback mechanism relied on the hypothalamic hormones and peripheral hormones secreted from 25 the target endocrine glands. A variety of receptor proteins present in the pituitary gland play a major role for regulating the hypothalamo-hypophysial system.

It has been widely known that these hormones, factors and receptors are widely distributed in the brain instead of 30 existing only locally in the hypothalamo-hypophysial system. This fact suggests that the substances which are called "hypothalamic hormones" are working as neurotransmitters or neuroregulators in the central nervous system. It is further considered that these substances are similarly 35 distributed even in the peripheral tissues to play the role of important functions. The pancreas plays an important role of carrying out the carbohydrate metabolism by secreting not only

a digestive fluid but also glucagon and insulin. Insulin is secreted from the β cells and its secretion is promoted chiefly by glucose. It has, however, been known that a variety of receptors exist in the β cells, and the secretion of 5 insulin is controlled by various factors such as peptide hormones (galanin, somatostatin, gastric inhibitory polypeptide, glucagon, amylin, etc.), sugars (mannose, etc.), amino acids, and neurotransmitters in addition to glucose.

It has thus been known that in the pituitary gland 10 and in the pancreas are present receptor proteins for many hormones and neurotransmitters, said receptor proteins playing important roles for regulating the functions. As for the galanin and amylin, however, there has not yet been reported any discovery concerning the structure of their receptor 15 protein cDNAs. It is not known whether there exist any unknown receptor proteins or receptor protein subtypes.

For substances regulating the functions of the pituitary gland and pancreas, there exist receptor proteins specific to said substance on the surfaces of various 20 functional cells of the pituitary gland and pancreas. The pituitary gland and the pancreas are associations of a plurality of functional cells, and the actions of the individual substances are defined by the distributions of their target receptor proteins among the functional cells. 25 Accordingly, a substance, in many cases, exhibits an extensive variety of actions. To comprehend such complex systems, it is necessary to clarify the relations between the acting substances and the specific receptor proteins. It is further necessary to efficiently screen for receptor protein agonists 30 and antagonists capable of regulating the pituitary gland and pancreas, to clarify the structures of genes of receptor proteins from the standpoint of investigating and developing pharmaceuticals, and further to express them in a suitable expression system.

35 By utilizing the fact that a G protein coupled receptor protein exhibits homology in part of the structure thereof at the amino acid sequence level, an experiment of

looking at DNAs coding for novel receptor proteins relying upon a polymerase chain reaction (hereinafter simply referred to as "PCR") has recently been made.

In the central nervous system, many receptor proteins such as dopamine receptor protein, LH-RH receptor protein, neurotensin receptor protein, opioid receptor protein, CRF receptor protein, CRF receptor protein, somatostatin receptor protein, galanin receptor protein, TRH receptor protein, etc. are G protein coupled receptor proteins, and it has been clarified that ligands to these receptors exert a variety of effects in the central nervous system.

In the immune system, an α - or a β -chemokine receptor protein, an MIP1 α receptor protein, an IL-8 receptor protein, a C5a receptor protein, etc. have been known as such G protein coupled receptor proteins, and are working as receptor proteins responsive to immunoregulating substances to play important roles for regulating the functions of the living body. There is, for example, an IL-6 receptor protein that acts both in the above-mentioned central nervous system and in the immune system. IL-6 is both a β -cell differentiating factor and a biologically active factor related to the proliferation and differentiation of nerve cells.

It has been widely known that these hormones, factors and receptor proteins are usually widely distributed up to the peripheral tissues instead of existing only locally in the central nervous system and in the immune system and are producing important functions, respectively. Agonists and antagonists for these receptor proteins are now being developed as various useful pharmaceuticals.

For substances regulating the functions of the central nervous system and the immune system, there exist receptor proteins specific to said substance on the surfaces of various functional cells of the central nervous system and the immune system. The central nervous system and the immune system are associations of a plurality of functional cells, and the actions of the individual substances are defined by the distributions of their target receptor proteins among the

functional cells. Accordingly, a substance, in many cases, exhibits an extensive variety of actions. Moreover, there is an example wherein many factors play a part in a physiological phenomenon. To comprehend such complex systems, 5 it is necessary to clarify relations between the acting substances and the specific receptor proteins.

As discussed herein above, the G protein coupled receptor protein is present on the cell surface of living body cells and organs and has a very important role as a target for 10 molecules such as hormones, neurotransmitters and physiologically active substances, which molecules control, regulate or adjust the functions of living body cells and organs.

SUMMARY OF THE INVENTION

15 One object of the present invention is to provide novel DNAs which are useful as DNA primers for a polymerase chain reaction; methods for amplifying a DNA coding for a G protein coupled receptor protein using said DNA; screening methods for the DNA coding for a G protein coupled receptor 20 protein using said DNA; DNAs obtained by said screening method; and G protein coupled receptor proteins encoded by the DNA obtained by said screening method, peptide fragments or segments thereof, modified peptide derivatives thereof or salts thereof.

25 Another object of the present invention is to provide processes for producing said receptor protein; transformants capable of expressing said receptor protein; cell membrane fractions obtained from said transformant; methods for determining a ligand to the receptor protein; screening methods 30 for a compound or a salt thereof capable of inhibiting the binding of the ligand with the receptor protein; kits for said screening method, pharmaceutical compositions comprising an effective amount of the inhibitory compound; antibodies against said receptor protein; immunoassays using said receptor 35 protein or said antibody and use of said receptor protein and encoding DNA.

Yet another object of the present invention is to provide novel G protein coupled receptor proteins which are expressed in pituitary glands or pancreatic β cells; DNAs comprising a DNA coding for said G protein coupled receptor protein; processes for producing said receptor protein; transformants capable of expressing said receptor protein; cell membrane fractions obtained from said transformant; methods for determining a ligand to the receptor protein; screening methods for a compound or a salt thereof capable of inhibiting the binding of the ligand with the receptor protein; kits for said screening method, pharmaceutical compositions comprising the inhibitory compound; antibodies against said receptor protein; immunoassays using said receptor protein or said antibody and use of said receptor protein and encoding DNA.

Still another object of the present invention is to provide novel human amygdaloid nucleus-derived G protein coupled receptor proteins; DNAs comprising a DNA coding for said G protein coupled receptor protein; processes for producing said receptor protein; transformants capable of expressing said receptor protein; cell membrane fractions obtained from said transformant; methods for determining a ligand to the receptor protein; screening methods for a compound or a salt thereof capable of inhibiting the binding of the ligand with the receptor protein; kits for said screening method, pharmaceutical compositions comprising the inhibitory compound; antibodies against said receptor protein; immunoassays using said receptor protein or said antibody and use of said receptor protein and encoding DNA.

Yet another object of the present invention is to provide novel mouse pancreatic β cell line MIN6-derived G protein coupled receptor proteins; DNAs comprising a DNA coding for said G protein coupled receptor protein; processes for producing said receptor protein; transformants capable of expressing said receptor protein; cell membrane fractions obtained from said transformant; methods for determining a ligand to the receptor protein; screening methods for a

compound or a salt thereof capable of inhibiting the binding of the ligand with the receptor protein; kits for said screening method, pharmaceutical compositions comprising the inhibitory compound; antibodies against said receptor protein; 5 immunoassays using said receptor protein or said antibody and use of said receptor protein and encoding DNA.

The present inventors have succeeded in synthesizing novel DNA primers based upon the similarity (homology) with the base sequences coding for the first membrane-spanning region 10 or the sixth membrane-spanning region each of known G protein coupled receptor proteins. It is to be particularly noted that there has been no report of a DNA primer pair which has been synthesized paying attention to the similarity with the base sequence coding for the first and the sixth membrane-spanning 15 region of the known G protein coupled receptor protein.

Next the present inventors have succeeded in synthesizing other novel DNA primers based upon the similarity (homology) with the base sequences coding for the third or the sixth membrane-spanning region each of known G protein 20 coupled receptor proteins. They have also unexpectedly succeeded in efficiently amplifying DNAs (DNA fragments) coding for G protein coupled receptor proteins by means of PCR using those DNA primers.

They have further succeeded in synthesizing novel 25 DNA primers based upon the similarity (homology) with the base sequences coding for the second or the seventh membrane-spanning region each of known G protein coupled receptor proteins; upon the similarity (homology) with the base sequences coding for first or the third membrane-spanning 30 region each of known G protein coupled receptor proteins; and upon the similarity (homology) with the base sequences coding for the second or the sixth membrane-spanning region each of known G protein coupled receptor proteins. They have furthermore and unexpectedly succeeded in efficiently 35 amplifying DNAs (DNA fragments) coding for G protein coupled receptor proteins by conducting PCR using those DNA primers.

Moreover, the present inventors have succeeded in

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efficiently cloning full-length DNA coding for said G protein coupled receptor protein via using amplified DNAs (DNA fragments) coding for said G protein coupled receptor protein. Thus, they have found that novel DNA coding for novel G protein 5 coupled receptor proteins can be isolated, characterized or prepared via conducting amplifications and analyses of various DNA using said DNA primers.

To be more specific, the present inventors have selected amino acid sequences which are each common to the 10 portion corresponding to or near the first and the sixth membrane-spanning region of the known individual G protein coupled receptor proteins and have designed the DNA primer (SEQ ID NO: 1) coding for the amino acid sequence common (homologous) to the first membrane-spanning region and the DNA 15 primer (SEQ ID NO: 2) which is complementary to the nucleotide sequence coding for the amino acid sequence common (homologous) to the area near the sixth membrane-spanning region. Those DNA primers have a different nucleotide sequence as compared with reported DNA primers (e.g. a set of synthetic DNA 20 primers corresponding to the third and the sixth membrane-spanning regions (SEQ ID NO: 60 and SEQ ID NO: 61) as reported by Libert, F. et al.) and such instant primers are novel and unique.

Especially for an object of conducting an efficient 25 elongation reaction in the PCR, the 3'-terminal region of the instant primers contains the nucleotide sequence which is common (homologous) among many receptor proteins. Even in other areas, the similarity (homology) at the 30 nucleotide sequence level (base sequence level) is utilized for setting the mixed base (nucleotide) parts wherein their nucleotide sequences (base sequences) are matched for as many nucleotides (bases) as possible among many DNA for the receptor proteins. Then the present inventors have amplified cDNA derived from human brain amygdala, human pituitary gland and 35 rat brain, found the amplified products as shown in Figure 17 and, from those products, obtained the G protein coupled receptor protein cDNAs having the sequence as shown in

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Figure 18, Figure 19, Figure 20, Figure 21, Figure 22,
Figure 23, Figure 27, Figure 29, Figure 34, Figure 37,
Figure 40, Figure 43 or Figure 46. Among them, the G protein
coupled receptor protein cDNAs having the sequence as shown in
5 Figure 22, Figure 23, Figure 27, Figure 29, Figure 34,
Figure 37, Figure 40, Figure 43 or Figure 46 are novel.

Further, the present inventors have selected the
amino acid sequences common (homologous) to the third and the
sixth membrane-spanning region each of the known G protein
10 coupled receptor proteins and designed the DNA primers coding
for the amino acid sequence common (homologous) to the third
membrane-spanning region (SEQ ID NO: 3; SEQ ID NO: 5, SEQ ID
NO: 6 and SEQ ID NO: 7) and the DNA primers which are
complementary to the nucleotide sequence coding for the amino
15 acid sequence common (homologous) to the portion near the sixth
membrane-spanning region (SEQ ID NO: 4, SEQ ID NO: 8 and SEQ ID
NO: 9). Again, those DNA primers have different base sequences
from those of the DNA primers previously reported (e.g., a set
of synthetic DNA primers corresponding to the sequence of the
20 third and the sixth membrane-spanning regions (SEQ ID NO: 60
and SEQ ID NO: 61) as reported by Libert, F. et al.) and such
instant primers are novel and unique. The present inventors
amplified cDNA derived from the smooth muscles of gastric
pylorus of rabbits using said DNA primer and obtained G protein
25 coupled receptor protein cDNA having the sequence of Figure 49
or Figure 52. Those cDNAs are novel.

Still further, the present inventors have selected
the amino acid sequences common (homologous) to the second and
the seventh membrane-spanning region each of the known G
30 protein coupled receptor proteins and designed the DNA primer
coding for the amino acid sequence common (homologous) to the
second membrane-spanning region (SEQ ID NO: 10) and the DNA
primer which is complementary to the base sequence coding for
the amino acid sequence common (homologous) to the portions
35 near the seventh membrane-spanning region (SEQ ID NO: 11).
Those DNA primers have different base sequences from those of
DNA primers previously reported (e.g., a set of synthetic DNA

primers corresponding to the part of the third and the sixth membrane-spanning regions (SEQ ID NO: 60 and SEQ ID NO: 61) as reported by Libert, F. et al) and such instant primers are novel and unique. The present inventors amplified cDNA derived from the smooth muscles of gastric pylorus of rabbits using said DNA primer and obtained G protein coupled receptor protein cDNAs having each the sequence of Figure 55, Figure 56, Figure 72, or Figure 73. Those cDNAs are novel.

Furthermore, the present inventors have selected the amino acid sequences common (homologous) to the first and the third membrane-spanning region each of the known G protein coupled receptor proteins and designed the DNA primer coding for the amino acid sequence common (homologous) to the first membrane-spanning region (SEQ ID NO: 12) and the DNA primer which is complementary to the base sequence coding for the amino acid sequence common (homologous) to the portions near the third membrane-spanning region (SEQ ID NO: 13). Still further, the present inventors have selected the amino acid sequences common (homologous) to the third and the sixth membrane-spanning region each of the known G protein coupled receptor proteins and designed the DNA primers coding for the amino acid sequence common (homologous) to the third membrane-spanning region (SEQ ID NO: 10 and SEQ ID NO: 18) and the DNA primers which are complementary to the base sequence coding for the amino acid sequence common (homologous) to the parts near the sixth membrane-spanning region (SEQ ID NO: 15 and SEQ ID NO: 19). Further, the present inventors have selected the amino acid sequences common (homologous) to the second and the sixth membrane-spanning region each of the known G protein coupled receptor proteins and designed the DNA primer coding for the amino acid sequence common (homologous) to the second membrane-spanning region (SEQ ID NO: 16) and the DNA primer which is complementary to the base sequence coding for the amino acid sequence common (homologous) to the parts near the sixth membrane-spanning region (SEQ ID NO: 17). Those DNA primers have different base sequences from those of DNA primers previously reported (e.g., a set of synthetic DNA primers

corresponding to the part of the third and the sixth membrane-spanning regions (SEQ ID NO: 60 and SEQ ID NO: 61) as reported by Libert, F. et al) and such instant primers are novel and unique.

5 Still another object of the present invention is to provide a G protein coupled receptor protein expressed in the pituitary gland and pancreatic β cells, a DNA comprising a DNA coding for said protein, a process for producing said protein, and use of said protein and DNA.

10 In order to achieve the above-mentioned aims, the present inventors have made extensive investigations. As a result, the present inventors have succeeded in amplifying cDNA derived from the human pituitary gland and the mouse pancreatic β -cell strain, MIN 6, with a synthetic DNA primer for efficiently isolating G protein coupled receptor protein-encoding DNA, and have forwarded the analysis. Thus, the present inventors have succeeded in isolating novel human and mouse-derived G protein coupled receptor protein-encoding cDNAs, in determining the partial structure thereof, and have 15 considered that these cDNA sequences are preserved very well in the human and in the mouse, and are coding for novel receptor proteins for the same ligand. Based upon the above knowledge, the present inventors have discovered that these DNAs make it possible to obtain a cDNA having a full length 20 open reading frame (ORF) of the receptor protein, hence, to produce the receptor protein. The inventors have further discovered that the above-mentioned receptor protein obtained when the G protein coupled receptor protein-encoding cDNA is 25 expressed by a suitable means permits screening for a ligand to the receptor protein from the living body or from natural or non-natural compounds under guidance of data obtainable in receptor coupling tests or measurements of intracellular second messengers, etc. and further allows screening for a compound that inhibits the binding of the ligand and the 30 receptor protein.

In one embodiment, the present inventors have carried out PCR amplification of novel human pituitary gland-

derived cDNA fragments as shown in Figures 22 and 23, and have subcloned them to obtain a plasmid vector (p19P2). From analysis of the partial sequence, it has been clarified that the cDNA has been encoded a novel receptor protein.

5 The synthetic DNA primers used for amplifying the cDNA are corresponding to seven hydrophobic clusters that exist in the known G protein coupled receptor proteins in common, i.e., corresponding to the first and sixth membrane-spanning regions among the membrane-spanning domains. The nucleotide sequence

10 (SEQ ID NO: 29) has been determined from the primer region at the 5' side (first membrane-spanning domain side) and has been translated into an amino acid sequence (SEQ ID NO: 24) [Figure 22]. As a result, the second and third membrane-spanning domains have been confirmed on the hydrophobicity

15 plotting [Figure 58]. Similarly, the nucleotide sequence (SEQ ID NO: 30) has been determined from the primer region at the 3' side (sixth membrane-spanning domain side) and has been translated into an amino acid sequence (SEQ ID NO: 25) [Figure 23]. As a result, the presence of the sixth and fifth

20 membrane-spanning domains has been confirmed on the hydrophobicity plots [Figure 59]. The size of the amplified cDNA is about 700 bp which is nearly comparable with the number of bases between the first membrane-spanning domain and the sixth membrane-spanning domain of the known G protein coupled

25 receptor protein.

G protein coupled receptor proteins exert common property to some extent at an amino acid sequence level, and are forming one protein family. Therefore, data base retrieval has been carried out based upon the amino acid sequence of the 30 subject novel receptor protein (protein encoded by cDNA included in p19P2). As a result, a high homology has been exhibited as compared with the known G protein coupled receptor protein (rat neuropeptide Y receptor protein encoded by S12863) that is shown in Figure 60. This fact tells that 35 the novel receptor protein of the present invention belongs to the G protein coupled receptor protein family. Moreover, the data base has been retrieved using, as a template, the amino

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acid sequence encoded by the DNA of the invention. It exhibits high homology to the amino acid sequences of the known G protein coupled receptor proteins, mouse-derived ligand unknown RP-23 (B40470), human-derived ligand unknown K-opioid receptor 5 protein (P30098) and human-derived NK-2 receptor protein (JQ1059). However, none of them are in perfect agreement, from which it is learned that a novel receptor protein had been encoded. The aforementioned abbreviations in parentheses are reference numbers that are assigned when they are 10 registered as data to NBRF-PIR/Swiss-PROT and are, usually, each called "Accession Number".

Next, by using the novel G protein coupled receptor protein-encoding cDNA fragment (p19P2) of the present invention, a cDNA having a full-length open reading frame of 15 the receptor protein of the present invention has been obtained from human pituitary gland cDNA libraries. The nucleotide sequence analysis of a plasmid (phGR3) carrying the cDNA having a full length open reading frame of the receptor protein shows that the nucleotide sequence of a coding region of this 20 receptor protein is represented by SEQ ID NO: 31, and the amino acid sequence deduced therefrom is represented by SEQ ID NO: 26 [Figure 34]. Based upon the amino acid sequence, hydrophobicity plotting has been carried out. The results are shown in Figure 36. From the hydrophobicity plotting, it 25 has been clarified that the receptor protein of the present invention possessed seven hydrophobic domains. That is, it has been confirmed that the receptor protein encoded by the cDNA obtained according to the present invention is a seven transmembrane (membrane-spanning) G protein coupled 30 receptor protein. An expression of mRNA for receptor genes encoded by the cDNA of the present invention has been checked by northern blotting techniques at a mRNA level, and it has been confirmed that the receptor gene has been expressed in the human pituitary gland [Figure 35].

35 The present inventors have further succeeded in PCR amplification of a mouse pancreatic β cell strain, MIN6 derived cDNA fragment, and cloning of pG3-2 and pG1-10.

Then, based on the nucleotide sequence of cDNA included in these two plasmid vectors, the nucleotide sequence shown in Figure 27 has been derived. It was learned from the nucleotide sequence that the cDNA encodes a novel receptor protein.

5 Upon translating the nucleotide sequence into an amino acid sequence, the presence of the third, fourth, fifth and sixth membrane-spanning domains has been confirmed on the hydrophobicity plots [Figure 28]. The size of the amplified cDNA is about 400 bp which is nearly comparable with the number

10 of bases between the third membrane-spanning domain and the sixth membrane-spanning domain of the known G protein coupled receptor protein. The amino acid sequence has been compared with amino acid sequences [Figures 22 and 23] encoded by the G protein coupled receptor protein cDNA included in p19P2 cloned

15 from the human pituitary gland. As a result, homology is more than 95% [Figure 61]. From this fact, it was estimated that the protein encoded by the cDNA included in pG3-2 is a mouse type G protein coupled receptor protein relative to the human-derived one encoded by the cDNA included in p19P2.

20 The present inventors have further amplified a mouse pancreatic β -cell strain, MIN6-derived cDNA fragment by the PCR followed by subcloning into a plasmid vector to obtain a clone (p5S38) having a nucleotide sequence as shown in Figure 62. From the nucleotide sequence (SEQ ID NO: 33), it

25 has been clarified that the cDNA encodes a novel receptor protein. Upon translating the nucleotide sequence into an amino acid sequence (SEQ ID NO: 28), the presence of the third, fourth, fifth and sixth membrane-spanning domains has been confirmed on the hydrophobicity plots [Figure 64]. The size of

30 the amplified DNA is about 400 bp that is nearly comparable with the known G protein coupled receptor protein. The amino acid sequence has been compared with amino acid sequences [Figures 22 and 23] encoded by the G protein coupled receptor protein cDNA included in p19P2 cloned from the human pituitary

35 gland, and with amino acid sequences of proteins encoded by pG3-2 and pG1-10 derived from the mouse pancreatic β -cell strain. As a result, homology is more than 95% to them

[Figure 63]. This fact suggests that the protein encoded by the human-derived pituitary gland-derived p19P2, the proteins encoded by the mouse pancreatic β -cell strain-derived pG3-2 and pG1-10, and the protein encoded by the mouse pancreatic β -cell strain-derived p5S38, pertain to a receptor family that recognizes the same ligand.

Another object of the present invention is to provide a novel human amygdaloid nucleus-derived protein coupled receptor protein, a DNA containing a DNA coding for said G protein coupled receptor protein, a process for producing said G protein coupled receptor protein, and use of said protein and DNA.

The present inventors have synthesized DNA primers for efficiently isolating a DNA coding for G protein coupled receptor proteins, amplified an amygdaloid nucleus-derived cDNA with the above primer, and have analyzed it.

As a result, the present inventors have succeeded in isolating, from the human amygdaloid nucleus, a cDNA coding for a novel G protein coupled receptor protein and have determined its partial structure. The nucleotide sequence of the isolated cDNA is preserved very well as compared with that of the mouse glucocorticoid-induced receptor (hereinafter sometimes referred to as "GIR") and is considered to be encoding a receptor protein to the same ligand (Molecular Endocrinology 5:1331-1338, 1991). It is reputed that, in the mouse, the GIR is a receptor which is induced by glucocorticoid and expressed in T-cells and is working as a receptor to immunoregulating factors in the immune system on the T-cells. The present inventors have succeeded in the isolation of this human type GIR from the human amygdaloid nucleus. Accordingly, it is suggested that the isolated GIR is expressed even in the human central nervous system to carry out some function. From these facts, it is considered that the receptor protein is strongly expressed in the human brain and in the immune system and is also functioning therein. These characterized DNAs allow one to obtain a cDNA having a full length open reading frame of the receptor and production of the receptor

proteins. The receptor proteins expressed by a suitable means, furthermore, permit screening for a ligand to the receptor proteins from the living body or from natural and non-natural compounds depending on indications obtainable in 5 receptor protein-binding experiments, measurements of intracellular second messengers, etc. It further allows one to screen for compounds capable of inhibiting the binding between the ligand and the receptor protein.

To be more specific, the present inventors have 10 amplified, as a novel human amygdaloid nucleus-derived cDNA, one species, as shown in Figures 29 and 30, by PCR, cloned it, and clarified from the analysis of a partial sequence thereof that a novel receptor protein is encoded. The synthetic DNA primers used for amplifying the cDNA are corresponding to seven 15 hydrophobic clusters that exist in the G protein coupled receptor proteins in common, i.e., corresponding to the first and sixth membrane-spanning regions among the membrane-spanning domains. The nucleotide sequence has been determined from the primer region at the 5' side (first membrane-spanning domain 20 side) and has been translated into an amino acid sequence. As a result, the second and third membrane-spanning domains have been confirmed on the hydrophobicity plotting [Figure 31]. Similarly, the nucleotide sequence has been determined from the 25 primer region at the 3' side (sixth membrane-spanning domain side) and has been translated into an amino acid sequence . As a result, the presence of the fifth and fourth membrane- spanning domains has been confirmed on the hydrophobicity plots [Figure 32]. The size of the amplified cDNA is about 30 700 bp which is nearly comparable with the number of bases of the known G protein coupled receptor protein.

The inventors have further retrieved the data base based on, as a template, the nucleotide sequence of the isolated DNA and observed high homology to the DNA that codes for mouse-derived glucocorticoid-induced receptor protein which 35 is a widely known G protein coupled receptor protein [Figure 33]. This result strongly suggests that the DNA of the present invention is encoding a human-type receptor protein of GIR.

Yet another object of the present invention is to provide a novel mouse pancreatic β -cell strain, MIN6-derived protein coupled receptor protein, a DNA containing a DNA coding for said G protein coupled receptor protein, a process 5 for producing said G protein coupled receptor protein, and use of said protein and DNA. The present inventors have synthesized DNA primers for efficiently isolating a DNA coding for G protein coupled receptor proteins, amplified a mouse pancreatic β -cell strain, MIN6-derived cDNA with the above 10 primer, and have analyzed it.

As a result, the present inventors have succeeded in isolating a mouse-derived cDNA coding for a novel G protein coupled receptor protein and have determined its partial structure. The isolated cDNA is homologous to known G protein 15 coupled receptors at the nucleotide sequence level and at the amino acid sequence level and is considered to be encoding a novel receptor protein which is expressed in the mouse pancreas and is also functioning therein. These characterized DNAs allow one to obtain a cDNA having a full length open reading 20 frame of the receptor and production of the receptor proteins. Human-derived cDNAs may be cloned by using, as a probe, said mouse-derived cDNA. The receptor proteins expressed by a suitable means, furthermore, permit screening 25 for a ligand to the receptor protein from the living body or from natural and non-natural compounds relying on indications obtainable in receptor protein-binding experiments, measurements of intracellular second messengers, etc. It further allows one to screen for compounds capable of inhibiting the binding of the ligand with the receptor protein.

To be more specific, the present inventors have amplified, as a novel mouse pancreatic β -cell strain, MIN6-derived cDNA, p3H2-17, as shown in Figures 37, by PCR, cloned it, and clarified from the analysis of a partial sequence thereof that a novel receptor protein is encoded. The 35 nucleotide sequence has been translated into an amino acid sequence. As a result, the presence of the third, fourth, fifth and sixth membrane-spanning domains has been confirmed

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on the hydrophobicity plots [Figure 38]. The size of the amplified cDNA is about 400 bp which is nearly comparable with that of the known G protein coupled receptor protein.

The inventors have retrieved the data base based
5 on, as a template, the nucleotide sequence of the isolated DNA
and observed 30% homology to chicken ATP receptor (P34996),
25% homology to human somatostatin receptor subtype 3 (A46226),
27% homology to human somatostatin receptor subtype 4 (JN0605),
and 28% homology to bovine neuropeptide Y receptor (S28787),
10 respectively (Figure 39), which are known G protein coupled
receptor proteins. The aforementioned abbreviations in
parentheses are reference numbers that are assigned when they
are registered as data to NBRF-PIR/Swiss-PROT and are, usually,
each called "Accession Number".

15 An expression of receptor genes encoded by the cDNA
fragment included in p3H2-17 of the present invention has been
checked by northern blotting techniques at a mRNA level, and it
has been confirmed that the receptor gene has been intensely
expressed in the mouse thymus and spleen. It has been also
20 confirmed that the receptor gene has been expressed in the
mouse brain and pancreas (Figure 65).

Next, by utilizing the information on the nucleotide
sequence of the fragment included in p3H2-17, cDNA encoding
a full-length open reading frame of the mouse pancreatic
25 β -cell strain, MIN6-derived G protein coupled receptor protein
of the present invention has been obtained from mouse
thymic and spleenic poly(A)⁺ RNA by 5'RACE (5' rapid
amplification of cDNA ends) techniques (Frohman M.A. et al.,
Proc. Natl. Acad. Sci. USA, 85:8998-9002 (1988); Belyavsky A.
30 et al., Nucleic Acids Res., 17:2919-2932 (1989); Edwards
J.B.D.M. et al., Nucleic Acids Res., 19:5227-5232 (1991)) and
3'RACE (3' rapid amplification of cDNA ends) techniques
(Frohman M.A. et al., Proc. Natl. Acad. Sci. USA, 85:8998-9002
(1988); Belyavsky A. et al., Nucleic Acids Res., 17:2919-2932
35 (1989)).

The plasmid (pMAH2-17) carrying cDNA encoding a
full-length open reading frame of the receptor protein of the

present invention has been subjected to sequencing analysis. As a result, the nucleotide sequence of the region coding for the receptor protein is represented by SEQ ID NO: 41 and the amino acid sequence deduced therefrom is represented by SEQ ID NO: 39 (Figure 69). Based on the amino acid sequence, hydrophobicity plotting has been carried out. The results are shown in Figure 70.

It has been clarified from the hydrophobicity plotting that the mouse pancreatic β -cell strain, MIN6-derived receptor protein of the present invention has seven hydrophobic domains. Thus, it has been confirmed that the receptor protein encoded by the cDNA included in pMAH2-17 according to the present invention is a seven transmembrane G protein coupled receptor protein.

Data base retrieval has been carried out based on the full-length amino acid sequence encoded by the cDNA included in pMAH2-17, and it has been observed that the amino acid sequence has 44.0% homology to mouse P_{2U} purinoceptor (P35383) and 38.1% homology to chicken P_{2Y} purinoceptor (P34996), respectively (Figure 71), which are known G protein coupled receptor proteins. The aforementioned abbreviations in parentheses are reference numbers that are assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are, usually, each called "Accession Number".

Since the receptor protein encoded by pMAH2-17 is highly homologous to prinoceptors, it is considered that there are strong possibility of a subtype within prinoceptor families. Therefore, the present inventors have carried out an electrophysiological analysis of the receptor gene in Xenopus oocytes and found significant inward currents elicited by Xenopus oocytes carrying the subject receptor gene in response to ATP stimulation (Figure 75). As a result, it has been determined that the receptor encoded by pMAH2-17 is one of the subtypes within prinoceptor families. It has been discussed and expected that there are a variety of subtypes among purinoceptors (Pharmac. Ther., Vol. 64, pp. 445-475 (1994)).

All data are supporting that the mouse pancreatic β -cell strain, MIN6-derived receptor protein of the present invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by pMAH2-17) is a novel purinoceptor subtype which is
5 clearly distinct from chicken P_{2y1} purinoceptor (FEBS LETTERS, Vol. 324(2), 219-225 (1993)); mouse P_{2y2} or P_{2u} purinoceptor (Proc. Natl. Acad. Sci. USA, Vol. 90, pp.5113-5117 (1993)); rat
 P_{2u} or P_{2y2} purinoceptor (Am. J. Respir. Cell Mol. Biol., Vol. 12, pp. 27-32 (1995)); human P_{2u} or P_{2y2} purinoceptor (Proc.
10 Natl. Acad. Sci. USA, Vol. 91, pp.3275-3279 (1994)); and rat P_{2x} purinoceptor (Nature, Vol. 371.6, pp.516-519 (1994)).

It is also strongly suggested that agonists and/or antagonists related to the receptor encoded by pMAH2-17 would be useful in therapeutic or prophylactic treatment of
15 diseases or syndromes in connection with purine ligand compounds. It is expected that the agonists of the receptor encoded by pMAH2-17 are useful as an immunomodulator or an antitumor agent, in addition they are useful in therapeutically or prophylactically treating hypertension, diabetes, cystic
20 fibrosis, etc. It is still expected that the antagonists of the receptor encoded by pMAH2-17 are useful as hypotensive agents, analgesics, agents for therapeutically or prophylactically treating incontinence of urine, etc.

Another object of the present invention is to
25 provide a novel human-derived protein coupled receptor protein of prinoceptor type, a DNA containing a DNA coding for said G protein coupled receptor protein, a process for producing said G protein coupled receptor protein, and use of said protein and DNA. The present inventors have synthesized DNA primers for
30 efficiently isolating a DNA coding for prinoceptor type G protein coupled receptor proteins on the basis of the nucleotide sequence of mouse purinoceptor, amplified a human-derived cDNA with the above primer, and have analyzed it.

As a result, the present inventors have succeeded in
35 isolating a human-derived cDNA coding for a novel G protein coupled receptor protein and have determined its full-length structure [Figure 77]. The isolated cDNA is homologous to

mouse G protein coupled receptor (purinoceptor) at the nucleotide sequence level and at the amino acid sequence level (87% homology; Figure 79) and is considered to be encoding a novel purinoceptor protein. The receptor proteins expressed by 5 a suitable means, furthermore, permit screening for a ligand to the receptor protein from the living body or from natural and non-natural compounds relying on indications obtainable in receptor protein-binding experiments, etc. It further allows one to screen for compounds capable of inhibiting the binding 10 of the ligand with the receptor protein.

It is also strongly suggested that agonists and/or antagonists related to the human receptor encoded by phAH2-17 would be useful in therapeutic or prophylactic treatment of diseases or syndromes in connection with purine ligand 15 compounds. It is expected that the agonists of the human receptor are useful as an immunomodulator or an antitumor agent, in addition they are useful in therapeutically or prophylactically treating hypertension, diabetes, cystic fibrosis, etc. It is still expected that the antagonists of 20 the human receptor are useful as hypotensive agents, analgesics, agents for therapeutically or prophylactically treating incontinence of urine, etc.

Accordingly, one aspect of the present invention is
(1) DNAs comprising a nucleotide sequence

25 represented by a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 19;

(2) DNAs according to the above (1) comprising a nucleotide sequence represented by a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 9;

30 (3) DNAs according to the above (1) comprising a nucleotide sequence represented by SEQ ID NO: 1 or SEQ ID NO: 2;

(4) DNAs according to the above (1) wherein the DNA is a primer for polymerase chain reaction in order to amplify 35 a DNA coding for a G protein coupled receptor protein;

(5) a method for amplifying a DNA coding for a G

protein coupled receptor protein by polymerase chain reaction techniques, which comprises:

(i) carrying out a polymerase chain reaction in the presence of a mixture of

5 ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

10 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

15 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19; or

20 (ii) carrying out a polymerase chain reaction in the presence of a mixture of

25 ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

30

35

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

5 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13;

(6) a method for screening a DNA library for

10 a DNA coding for a G protein coupled receptor protein, which comprises:

(i) carrying out a polymerase chain reaction in the presence of a mixture of

15 ① said DNA library,

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

20 25 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide

30 35

sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19,

5 to amplify selectively a template DNA coding for G protein coupled receptor protein, contained in the DNA library; or
(ii) carrying out a polymerase chain reaction in the presence of a mixture of

① said DNA library

10 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

15 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13,

 to amplify selectively a DNA coding for G protein coupled receptor protein, contained in the DNA library;

20 (7) a DNA coding for a G protein coupled receptor protein, which is obtained by a method according to the above (5) or (6); and

 (8) G protein coupled receptor proteins encoded by a DNA according to the above (7), their peptide segments or fragments and salts thereof.

 Another specific aspect of the invention is:

 (9) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the first to sixth membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

35 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers

comprising a nucleotide sequence represented by SEQ ID NO: 12, and

③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19;

15 (10) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the first to seventh membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11;

25 (11) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the third to sixth membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① a DNA coding for G protein coupled receptor protein,

said DNA being capable of acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19;

(12) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the third to seventh membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a

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nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

5 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11;

(13) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the second to sixth membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

10 ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
15 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers comprising a nucleotide sequence represented by SEQ ID

20 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID

25 NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19;

30 (14) a method for amplifying a DNA coding for G protein coupled receptor protein (e.g. a region corresponding to from the second to seventh membrane-spanning domains of G protein coupled receptor proteins or other domains thereof) by polymerase chain reaction techniques, which comprises carrying

out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein,
said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group
5 consisting of DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 10 and DNA primers
comprising a nucleotide sequence represented by SEQ ID
NO: 16, and
- ③ at least one DNA primer selected from the group
10 consisting of DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 11;

(15) a method for amplifying a DNA coding for G
protein coupled receptor protein (e.g. a region corresponding
to from the first to third membrane-spanning domains of G
15 protein coupled receptor proteins or other domains thereof) by
polymerase chain reaction techniques, which comprises carrying
out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein,
said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group
20 consisting of DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 1 and DNA
primers comprising a nucleotide sequence
represented by SEQ ID NO: 12, and
- ③ at least one DNA primer selected from the group
25 consisting of DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 13;

(16) a method for amplifying a DNA coding for G
protein coupled receptor protein by polymerase chain reaction
30 techniques, which comprises carrying out a polymerase chain
reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein,
said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group
35 consisting of DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 1, and
- ③ at least one DNA primer selected from the group

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consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2;

5 (17) a method for amplifying a DNA coding for G protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4;

15 (18) a method for amplifying a DNA coding for G protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8;

25 (19) a method for amplifying a DNA coding for G protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide

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sequence represented by SEQ ID NO: 11;

(20) a method for amplifying DNA coding for a G protein coupled receptor protein which comprises

(i) carrying out a polymerase chain reaction in the presence
5 of a mixture of

- ① a DNA coding for G protein coupled receptor protein,
said DNA being capable of acting as a template,
- ② at least one DNA primer which is capable of
binding with the 3'-side nucleotide sequence of
10 the - chain (minus chain) of the template DNA
coding for G protein coupled receptor protein to
allow the extension of the + chain (plus chain)
in the 5' → 3' direction, said DNA primer being
selected from the group consisting of DNA primers
15 comprising a nucleotide sequence represented by SEQ ID
NO: 1, DNA primers comprising a nucleotide sequence
represented by SEQ ID NO: 3, DNA primers comprising a
nucleotide sequence represented by SEQ ID NO: 5, DNA
primers comprising a nucleotide sequence represented by
SEQ ID NO: 6, DNA primers comprising a nucleotide
sequence represented by SEQ ID NO: 7, DNA primers
comprising a nucleotide sequence represented by
SEQ ID NO: 10, DNA primers comprising a
nucleotide sequence represented by SEQ ID NO: 12,
20 DNA primers comprising a nucleotide sequence
represented by SEQ ID NO: 14, DNA primers
comprising a nucleotide sequence represented by
SEQ ID NO: 16 and DNA primers comprising a
nucleotide sequence represented by SEQ ID NO: 18,
25 and
- ③ at least one DNA primer which is capable of
binding with the 3'-side nucleotide sequence of
the + chain (plus chain) of the template DNA
coding for G protein coupled receptor protein to
allow the extension of the - chain (minus chain)
30 in the 5' → 3' direction, said DNA primer being
selected from the group consisting of DNA primers

comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19,
or
(ii) carrying out a polymerase chain reaction in the presence of a mixture of
① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
② at least one DNA primer which is capable of binding with the 3'-side nucleotide sequence of the - chain (minus chain) of the template DNA coding for G protein coupled receptor protein to allow the extension of the + chain (plus chain) in the 5' → 3' direction, said DNA primer being selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and
③ at least one DNA primer which is capable of binding with the 3'-side nucleotide sequence of the + chain (plus chain) of the template DNA coding for G protein coupled receptor protein to allow the extension of the - chain (minus chain) in the 5' → 3' direction, said DNA primer being selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13;
(21) a method for screening DNA libraries for

a DNA coding for G protein coupled receptor protein (e.g. from the first to sixth membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a
5 mixture of

- ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19,
- 25 to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the first to sixth membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;
- (22) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein (e.g. from the first to seventh membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a mixture of
- 35 ①. said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide

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sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11,

5 to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the first to seventh membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

10 (23) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein (e.g. from the third to sixth membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a mixture of

15 ① said DNA library,

20 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide

sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by

25 SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

30 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide

sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA

primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19,

5 to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the third to sixth membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

(24) a method for screening DNA libraries for

10 a DNA coding for G protein coupled receptor protein (e.g. from the third to seventh membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a mixture of

15 ① said DNA library,

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by

20 SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

25 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11,

30 to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the third to seventh membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

(25) a method for screening DNA libraries for

35 a DNA coding for G protein coupled receptor protein (e.g. from the second to sixth membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises

carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19,

to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the second to sixth membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

(26) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein (e.g. from the second to seventh membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16, and

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③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11,
to amplify selectively a template DNA coding for G protein
5 coupled receptor protein (e.g. from the second to seventh membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

10 (27) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein (e.g. from the first to third membrane-spanning domains or other domains of G protein coupled receptor protein), which comprises carrying out a polymerase chain reaction in the presence of a mixture of

15 ① said DNA library,
② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

20 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13,
to amplify selectively a template DNA coding for G protein coupled receptor protein (e.g. from the first to third
25 membrane-spanning domains or other domains of G protein coupled receptor protein), contained in the DNA library;

30 (28) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

① said DNA library,
② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, and
35 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2,

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to amplify selectively the template DNA coding for G protein coupled receptor protein, contained in the DNA library;

5 (29) a method for screening DNA libraries to detect a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- 10 ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4,

15 to amplify selectively a template DNA coding for G protein coupled receptor protein, contained in the DNA library;

(30) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- 20 ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8,

25 to amplify selectively a template DNA coding for G protein coupled receptor protein, contained in the DNA library;

30 (31) a method for screening DNA libraries for a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

- 35 ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, and
- ③ at least one DNA primer selected from the group

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consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, to amplify selectively a template DNA coding for G protein coupled receptor protein, contained in the DNA library; and

5 (32) a method for screening DNA libraries according to any of the above (6), and (21) to (31) wherein said DNA library is derived from an origin selected from the group consisting of human tissues and human cells. Examples of such human tissues include adrenal, umbilical cord, brain, tongue, 10 liver, lymph gland, lung, thymus, placenta, peritoneum, retina, spleen, heart, smooth muscle, intestine, vessel, bone, kidney, skin, fetus, mammary gland, ovary, testis, pituitary gland, pancreas, submandibular gland, spine, prostate gland, stomach, thyroid gland, trachea (windpipe), skeletal muscle, uterus, 15 adipose tissue, urinary bladder, cornea, olfactory bulb, bone marrow, amnion, etc. Examples of such human cells include nerve cells, epithelial cells, endothelial cells, leukocytes, lymphocytes, gliocytes, fibroblasts, keratinized cells, osteoblasts, osteoclasts, astrocytes, melanocytes, various 20 carcinomas, various sarcomas, various cells derived from the above-mentioned human tissues.

Yet another aspect of the present invention is a degenerate deoxynucleotide which has an oligonucleotide sequence to which a SEQ ID NO selected from the group 25 consisting of SEQ ID NO: 1 to SEQ ID NO: 19 is assigned.

Another aspect of the present invention is
(33) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by SEQ ID NO: 24 and/or SEQ ID 30 NO: 25 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 24 or SEQ ID NO: 25; or a salt thereof;

(34) a G protein coupled receptor protein according to the above (33) comprising an amino acid sequence selected 35 from the group consisting of an amino acid sequence represented by SEQ ID NO: 26 and substantial equivalents to the amino

acid sequence represented by SEQ ID NO: 26; or a salt thereof;

(35) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 27 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 27; or a salt thereof;

(36) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 28 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 28; or a salt thereof;

(37) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of amino acid sequences represented by SEQ ID NO: 34 and/or SEQ ID NO: 35 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 34 or SEQ ID NO: 35; or a salt thereof;

(38) a G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 38 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 38; or a salt thereof;

(39) a G protein coupled receptor protein according to the above (38) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 39 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 39; or a salt thereof;

(40) a G protein coupled receptor protein comprising an amino acid sequence represented by SEQ ID NO: 56 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 56; or a salt thereof;

(41) a peptide segment or fragment of a G protein coupled receptor protein according to any of the above (33) to (40), a modified derivative thereof or a salt thereof;

(42) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (33);

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(43) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (34);

5 (44) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (35);

(45) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (36);

10 (46) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (37);

15 (47) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (38);

(48) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (39);

20 (49) a DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of the above (40);

(50) a DNA of the above (42) comprising a nucleotide sequence represented by SEQ ID NO: 29 and/or SEQ ID NO: 30;

25 (51) a DNA of the above (43) comprising a nucleotide sequence represented by SEQ ID NO: 31;

(52) a DNA of the above (44) comprising a nucleotide sequence represented by SEQ ID NO: 32;

(53) a DNA of the above (45) comprising a nucleotide sequence represented by SEQ ID NO: 33;

30 (54) a DNA of the above (46) comprising a nucleotide sequence represented by SEQ ID NO: 36 and/or SEQ ID NO: 37;

(55) a DNA of the above (47) comprising a nucleotide sequence represented by SEQ ID NO: 40;

35 (56) a DNA of the above (48) comprising a nucleotide sequence represented by SEQ ID NO: 41;

(57) a DNA of the above (49) comprising a nucleotide sequence represented by SEQ ID NO: 57;

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(58) a vector comprising a DNA according to any of the above (42) to (57);

(59) a transformant (including a transfectant) carrying a vector of the above (58);

5 (60) a process for producing a G protein coupled receptor protein or a salt thereof according to any of the above (33) to (40), which comprises culturing a transformant of the above (59) to express said G protein coupled receptor protein on the membrane of the transformant;

10 (61) a method for determining a ligand to a G protein coupled receptor protein according to any of the above (33) to (40), which comprises contacting

15 (i) at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof,

with

20 (ii) at least one compound to be tested;

(62) a screening method for a compound capable of inhibiting the binding of a G protein coupled receptor protein according to any of the above (33) to (40) with a ligand, which comprises carrying out a comparison between:

25 (i) at least one case where said ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof,

30 and

(ii) at least one case where said ligand together with a compound to be tested is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the

above (41), and mixtures thereof;

(63) a kit for the screening of one or more compounds capable of inhibiting the binding of a G protein coupled receptor protein according to any of the above (33) to (40), with a ligand, which comprises at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof; and

10 (64) an antibody against at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof.

15 Yet another aspect of the present invention is

(65) a G protein coupled receptor protein according to the above (33) comprising

20 (i) an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 24, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 24, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 24, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 24 are substituted with one or more other amino acid residues, or/and

25 (ii) an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 25, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 25, amino acid

sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 25, and amino acid sequences wherein one or 5 more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 25 are substituted with one or more other amino acid residues, or a salt thereof;

10 (66) a G protein coupled receptor protein according to the above (34) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 26, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 26, amino 15 acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 26, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 26 are substituted with 20 one or more other amino acid residues, or a salt thereof;

25 (67) a G protein coupled receptor protein according to the above (35) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 27, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 27, amino 30 acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 27, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in 35

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the amino acid sequence of SEQ ID NO: 27 are substituted with one or more other amino acid residues, or a salt thereof;

(68) a G protein coupled receptor protein according to the above (36) comprising an amino acid sequence selected

5 from the group consisting of an amino acid sequence represented by SEQ ID NO: 28, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 28, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 28, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 28 are substituted with one or more other amino acid residues, or a salt thereof;

(69) a G protein coupled receptor protein according to the above (37) comprising

20 (i) an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 34, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 34, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 34, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 34 are substituted with one or more other amino acid residues, or/and

25 (ii) an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 35, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more

preferably from 2 to 10 amino acid residues) are deleted
from the amino acid sequence of SEQ ID NO: 35, amino acid
sequences wherein one or more amino acid residues (preferably
from 2 to 30 amino acid residues, more preferably from 2 to
5 10 amino acid residues) are added to the amino acid sequence
of SEQ ID NO: 35, and amino acid sequences wherein one or
more amino acid residues (preferably from 2 to 30 amino acid
residues, more preferably from 2 to 10 amino acid residues)
in the amino acid sequence of SEQ ID NO: 35 are substituted
10 with one or more other amino acid residues,
or a salt thereof;

(70) a G protein coupled receptor protein according
to the above (38) comprising an amino acid sequence selected
from the group consisting of an amino acid sequence represented
15 by SEQ ID NO: 38, amino acid sequences wherein one or more
amino acid residues (preferably from 2 to 30 amino acid
residues, more preferably from 2 to 10 amino acid residues)
are deleted from the amino acid sequence of SEQ ID NO: 38,
amino acid sequences wherein one or more amino acid residues
20 (preferably from 2 to 30 amino acid residues, more preferably
from 2 to 10 amino acid residues) are added to the amino acid
sequence of SEQ ID NO: 38, and amino acid sequences wherein one
or more amino acid residues (preferably from 2 to 30 amino acid
residues, more preferably from 2 to 10 amino acid residues) in
25 the amino acid sequence of SEQ ID NO: 38 are substituted with
one or more other amino acid residues, or a salt thereof;

(71) a G protein coupled receptor protein according
to the above (39) comprising an amino acid sequence selected
from the group consisting of an amino acid sequence represented
30 by SEQ ID NO: 39, amino acid sequences wherein one or more
amino acid residues (preferably from 2 to 30 amino acid
residues, more preferably from 2 to 10 amino acid residues)
are deleted from the amino acid sequence of SEQ ID NO: 39,
amino acid sequences wherein one or more amino acid residues
35 (preferably from 2 to 30 amino acid residues, more preferably
from 2 to 10 amino acid residues) are added to the amino acid
sequence of SEQ ID NO: 39, and amino acid sequences wherein one

or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 39 are substituted with one or more other amino acid residues, or a salt thereof;

5 (72) a G protein coupled receptor protein according to the above (40) comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 56, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 56, amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid 10 sequence of SEQ ID NO: 56, and amino acid sequences wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 56 are substituted with one or more other amino acid residues, or a salt thereof;

15

20 (73) a method for determining a ligand according to the above (61) wherein said ligand is selected from the group consisting of angiotensin, bombesin, canavinoind, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive 25 intestinal and related peptides), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptides), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxanes, adenosine, adrenaline, α - and β -chemokine (IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , 30 MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neuropeptidin, TRH, pancreatic polypeptides and galanin;

35 (74) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to the said G protein coupled receptor

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protein in at least two cases:

- (i) where the labeled ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof, and
- 5 (ii) where the labeled ligand together with a compound to be tested is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof,
- 10 and comparing the measured amounts of the labeled ligand;
 - (75) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to a cell comprising the said G protein coupled receptor protein in at least two cases:
- 15
 - (i) where the labeled ligand is contacted with the said cell, and
 - (ii) where the labeled ligand together with a compound to be tested is contacted with the said cell,
- 20 and comparing the measured amounts of the labeled ligand;
 - (76) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to a membrane fraction of a cell comprising the said G protein coupled receptor protein in at least two cases:
- 25
 - (i) where the labeled ligand is contacted with the said membrane fraction, and
 - (ii) where the labeled ligand together with a compound to be tested is contacted with the membrane fraction,

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and comparing the measured amounts of the labeled ligand;

(77) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring amounts of a labeled ligand bound to said G protein coupled receptor protein in at least two cases:

10 (i) where the labeled ligand is contacted with a G protein coupled receptor protein according to any of the above (33) to (40) which is expressed on the membrane of a transformant according to the above (59) during incubation of the transformant, and

15 (ii) where the labeled ligand together with a compound to be tested is contacted with the G protein coupled receptor protein according to any of the above (33) to (40) which is expressed on the membrane of a transformant according to the above (59) during incubation of the transformant,

and comparing the measured amounts of the labeled ligand;

20 (78) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the above (33) to (40), which comprises measuring G protein coupled receptor protein-mediated cell-stimulating activities in at least two cases:

25 (i) where a compound capable of activating the G protein coupled receptor protein according to any of the above (33) to (40) is contacted with a cell comprising the said G protein coupled receptor protein, and

30 (ii) where the compound capable of activating the G protein together with a compound to be tested is contacted with the cell comprising the said G protein coupled receptor protein,

and comparing the measured cell-stimulating activities;

35 (79) a method for the screening of a compound or a salt thereof capable of inhibiting the binding of a ligand with a G protein coupled receptor protein according to any of the

above (33) to (40), which comprises measuring G protein coupled receptor protein-mediated cell-stimulating activities in at least two cases:

5 (i) where a compound capable of activating the G protein coupled receptor protein according to any of the above (33) to (40) is contacted with a G protein coupled receptor protein according to any of the above

10 (33) to (40) which is expressed on the membrane of a transformant according to the above (59) during incubation of the transformant, and

15 (ii) where the compound capable of activating the G protein together with a compound to be tested is contacted with the G protein coupled receptor protein according to any of the above (33) to (40) which is expressed on the membrane of a transformant according to the above (59) during incubation of the transformant,

and comparing the measured cell-stimulating activities;

20 (80) a method according to the above (78) or (79) wherein said compound capable of activating the G protein coupled receptor protein according to any of the above (33) to (40) is selected from the group consisting of angiotensin, bombesin, canavinoïd, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive intestinal and related peptides), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptides), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenaline, α - and β -chemokine (IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neuropeptid Y, TRH, pancreatic polypeptides and galanin;

25

30 (81) a compound which is determined through a method according to any of the above (62) and (74) to (80) or a salt thereof;

35 (82) a pharmaceutical composition comprising an

effective amount of a compound according to the above (81) or a salt thereof;

5 (83) a screening kit according to the above (63), comprising a cell comprising a G protein coupled receptor protein according to any of the above (33) to (40);

(84) a screening kit according to the above (63), comprising a membrane fraction derived from a cell comprising a G protein coupled receptor protein according to any of the above (33) to (40);

10 (85) a screening kit according to the above (63), comprising a cell of the (59) or (109) mentioned herein below;

(86) a screening kit according to the above (63), comprising a membrane fraction derived from a cell of the (59) or (109);

15 (87) a compound which is determined by means of a screening kit according to any of the above (63) and (83) to (86) or a salt thereof;

20 (88) a pharmaceutical composition comprising an effective amount of a compound according to the above (87) or a salt thereof; and

25 (89) a method for measuring at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof, which comprises contacting an antibody according to the above (64) with the component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide segments or salts thereof according to the above (41), and mixtures thereof.

Still another aspect of the present invention is

30 (90) a ligand to a G protein coupled receptor protein according to any of the above (33) to (40), which is determined through the following step of:
35 contacting (i) at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above

(33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof,

with (ii) at least one compound to be examined; and

5 (91) a compound capable of inhibiting the binding of a G protein coupled receptor protein according to any of the above (33) to (40) with a ligand, which is determined through carrying out a comparison between:

10 (i) at least one case where said ligand is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof, and

15 (ii) at least one case where said ligand together with a compound to be tested is contacted with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof.

Another aspect of the present invention is

20 (92) a recombinant G protein coupled receptor protein and a salt thereof which is obtained by the expression of a DNA according to any of the above (42) to (57), or a modified or fragmented derivative thereof;

25 (93) a method for amplifying a DNA coding for G protein coupled receptor protein by polymerase chain reaction techniques, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

30 (1) a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template, and

35 (2) at least one DNA primer selected from the group consisting of DNA primers comprising either SEQ ID NO: 1 or SEQ ID NO: 2; and

(94) a method for screening DNA libraries for

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a DNA coding for G protein coupled receptor protein, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

5 (1) said DNA library, and
 (2) at least one DNA primer selected from the group consisting of DNA primers comprising either SEQ ID NO: 1 or SEQ ID NO: 2,

to amplify selectively the DNA coding for G protein coupled receptor protein, contained in the DNA library.

10 Yet another aspect of the present invention is
 (95) a monoclonal antibody against at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof;

15 (96) a preparation of purified polyclonal antibodies against at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof;

20 (97) an immunoassay for detecting a G protein coupled receptor protein which comprising

25 (i) incubating a sample to be tested with an antibody according to the above (64) to allow formation of an antigen-antibody complex; and

30 (ii) detecting an antigen-antibody complex formed in step (i); and

35 (98) an immunoassay for detecting antibodies against a G protein coupled receptor protein which comprising

 (i) incubating a sample to be tested with at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof to allow formation of an antigen-antibody complex; and

(ii) detecting an antigen-antibody complex formed in step (a).

Still another aspect of the present invention is
5 (99) an antisense DNA or RNA which comprises a nucleotide sequence complementary to at least a portion of a DNA according to any of the above (42) to (57), said antisense DNA or RNA being hybridizable to said DNA according to any of the above (42) to (57);

10 (100) an antisense DNA or RNA according to the above (99) wherein said antisense DNA or RNA comprises the 5' end hairpin loop, 5' end 6-base-pair repeat, 5' end untranslated region, protein translation initiation site or codon, ORF translation initiation site or codon, 3'-untranslated region, 3' end palindrome region, or 3' end hairpin loop of a G protein
15 coupled receptor protein DNA according to any of the above (42) to (57);

(101) an antisense DNA or RNA according to the above (99) in a pharmaceutically acceptable carrier;
20 (102) an antisense DNA or RNA according to the above (99) comprising from 2 to 50 nucleotides;

25 (103) a method for modulating the activity of a G protein coupled receptor protein comprising contacting cells expressing the G protein coupled receptor protein with an antisense DNA or RNA according to the above (99);

30 (104) a method for producing an antibody against a G protein coupled receptor protein according to any of the above (33) to (40), which comprises administering to an individual at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof; and

35 (105) a method for producing a hybridoma which produces a monoclonal antibody against a G protein coupled receptor protein according to any of the above (33) to (40), which comprises

(i) immunizing an individual with at least one

component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of the above (33) to (40), peptide fragments or segments or salts thereof according to the above (41), and mixtures thereof;

5 (ii) immortalizing antibody producing cells from the immunized individual;

(iii) selecting an immortal cell which produces antibodies reactive with the G protein coupled receptor protein; and

10 (iv) growing said immortal cell.

Yet another aspect of the present invention is
(106) a PCR screening kit for a DNA (or nucleotide sequence) coding for G protein coupled receptor protein in a DNA library which comprises

15 (i) ① at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers

20 comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

25 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA

30 primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide

sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19; or

5 (ii) ① at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and

10 ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13;

 (107) a vector comprising the DNA according to the above (7);

15 (108) an expression system comprising an open reading frame (ORF) of DNA derived from a G protein coupled receptor protein DNA according to any of the above (7) and (42) to (57), wherein the ORF is operably linked to a control sequence compatible with a desired host cell;

20 (109) a transformant (including a transfectant) carrying a vector of the above (107) or an expression system of the above (108);

 (110) a process for producing a G protein coupled receptor protein or a salt thereof, which comprises culturing the transformant of the above (109) to express said G protein coupled receptor protein on the membrane of the transformant;

25 (111) a method for expressing a polypeptide of G protein coupled receptor protein, comprising:

 (a) providing a transformant of the above (59) or (109); and

30 (b) incubating the transformant under conditions which allow expression of the polypeptide of G protein coupled receptor protein;

 (112) a method for preparing a transformant according to the above (59) or (109), comprising:

 (a) providing a host cell capable of transformation;

(b) providing a vector according to the above (58) or (107) or an expression system according to the above (108); and

5 (c) incubating (a) with (b) under conditions which allow transformation of the host cell with the vector or the expression system;

(113) a pharmaceutical composition according to the above (82) or (88), comprising an effective amount of a compound according to the above (81) or (87) or a pharmaceutically acceptable salt thereof in admixture with a pharmaceutically acceptable carrier, excipient or diluent;

10 (114) the pharmaceutical composition according to the above (82) or (88), for inhibiting the binding of a G protein coupled receptor protein according to the present invention with a ligand;

15 (115) a method for inhibiting the binding of a G protein coupled receptor protein according to the present invention with a ligand in a medium which comprises contacting an effective amount of a compound according to the above (81) or (87) or a salt thereof with said medium;

20 (116) a method for modulating the activity of a G protein coupled receptor protein comprising contacting cells expressing the G protein coupled receptor protein with a an effective amount of a compound according to the above (81) or (87) or a salt thereof;

25 (117) the ligand according to the above (90) being labeled with a detectable reporter;

(118) the antibody according to the above (64) wherein the antibody is labeled with a detectable reporter;

30 (119) a pharmaceutical composition for controlling an expression of G protein coupled receptor protein, which comprises an effective amount of the antisense DNA according to the above (99), and

35 (120) a culture product produced by a transformant according to the above (59) or (109).

Yet another aspect of the present invention is

(121) a DNA according to the above (1) wherein the

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DNA is an oligonucleotide having from 8 to 60 base residues;
(122) a DNA according to the above (1) wherein the
DNA is synthetic;

5 (123) a DNA (or nucleotide sequence) coding for a G
protein coupled receptor protein or a fragment thereof, which
is obtained through the method according to any of the above
(5) to (32);

10 (124) a DNA (or nucleotide sequence) according to
the above (123), wherein said G protein coupled receptor
protein is selected from the group consisting of angiotensin
receptor, bombesin receptor, canavinoind receptor,
cholecystokinin receptor, glutamine receptor, serotonin
receptor, melatonin receptor, neuropeptide Y receptor, opioid
receptor, purine receptor, vasopressin receptor, oxytocin
receptor, VIP receptor (vasoactive intestinal and related
peptide receptor), somatostatin receptor, dopamine receptor,
motilin receptor, amylin receptor, bradykinin receptor, CGRP
receptor (calcitonin gene related peptide receptor),
adrenomedullin receptor, leukotriene receptor, pancreastatin
receptor, prostaglandin receptor, thromboxane receptor,
adenosine receptor, adrenaline receptor, α - and β -chemokine
receptor including IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78,
PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β ,
and RANTES receptors, endothelin receptor, enterogastrin
receptor, histamine receptor, neurotensin receptor, TRH
receptor, pancreatic polypeptide receptor, and galanin
receptor; and

20 (125) a culture product produced by a transformant
according to the above (59) or (109).

30 As used herein the term "substantial equivalent(s)"
means that the activity of the protein, e.g., nature of the
ligand binding activity, and physical characteristics are
substantially the same. Substitutions, deletions or
insertions of amino acids often do not produce radical changes
35 in the physical and chemical characteristics of a polypeptide,
in which case polypeptides containing the substitution,

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deletion, or insertion would be considered to be substantially equivalent to polypeptides lacking the substitution, deletion, or insertion. Substantially equivalent substitutes for an amino acid within the sequence may be selected from other 5 members of the class to which the amino acid belongs. The non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and 10 glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the community (homology) of the 15 sequence of 5' side synthetic DNA primers (HS-1) having a nucleotide sequence represented by SEQ ID NO: 1 with the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 2 depicts the community (homology) of the 20 sequence which is complementary to 3' side synthetic DNA primers (HS-2) having a nucleotide sequence represented by SEQ ID NO: 2 with the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 3 depicts the community (homology) of the 25 sequence of 5' side synthetic DNA primers (3A) having a nucleotide sequence represented by SEQ ID NO: 5 or 5' side synthetic DNA primers (3B) having a nucleotide sequence represented by SEQ ID NO: 6 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs 30 and genes.

Figure 4 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (3C) having a 35 nucleotide sequence represented by SEQ ID NO: 7 or 5' side synthetic DNA primers (3D) having a nucleotide sequence represented by SEQ ID NO: 3 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs

and genes.

Figure 5 depicts the community (homology) of the sequence (6A) which is complementary to 3' side synthetic DNA primers having a nucleotide sequence represented by SEQ ID NO: 8 or the nucleotide sequence (6B) which is complementary to 3' side synthetic DNA primers having a nucleotide sequence represented by SEQ ID NO: 9 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 6 depicts the community (homology) of the sequence (6C) which is complementary to 3' side synthetic DNA primers having a nucleotide sequence represented by SEQ ID NO: 4 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 7 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (T2A) having a nucleotide sequence represented by SEQ ID NO: 10 with the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 8 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (T7A) having a nucleotide sequence represented by SEQ ID NO: 11 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 9 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (TM1-A2) having a nucleotide sequence represented by SEQ ID NO: 12 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 10 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (TM3-B2) having a nucleotide sequence represented by SEQ ID NO: 13 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 11 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (TM3-C2) having a nucleotide sequence represented by SEQ ID NO: 14 relative to

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the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 12 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (TM6-E2) having a nucleotide sequence represented by SEQ ID NO: 15 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 13 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (TM2F18) having a nucleotide sequence represented by SEQ ID NO: 16 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 14 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (TM6R21) having a nucleotide sequence represented by SEQ ID NO: 17 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 15 depicts the community (homology) of the sequence of 5' side synthetic DNA primers (S3A) having a nucleotide sequence represented by SEQ ID NO: 18 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 16 depicts the community (homology) of the sequence which is complementary to 3' side synthetic DNA primers (S6A) having a nucleotide sequence represented by SEQ ID NO: 19 relative to the nucleotide sequence each of other G protein coupled receptor protein-encoding cDNAs and genes.

Figure 17 is the 1.2% agarose gel electrophoresis profile of cDNA products each obtained from human brain amygdala (1, 2, 7), human pituitary body (3, 4, 8) and rat brain (5, 6, 9) by PCR amplification using the synthetic DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and the synthetic DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, wherein lanes 1 to 6 show the results of when PCR is carried out under severe conditions as disclosed in Examples, lanes 7 to 9 show the results of when PCR is carried out under mild conditions, and M denotes a size

marker which is obtained by cutting λ -phage DNA with restriction enzyme, EcoT14I.

Figure 18 shows the nucleotide sequence determined by sequencing of clone A58 with a T7 primer wherein the 5 clone A58 is obtained by amplifying human brain amygdala-derived cDNA by PCR under mild conditions and subcloning it to pCRTM II.

Figure 19 shows the nucleotide sequence determined by sequencing of clone A58 with an SP6 primer.

10 Figure 20 shows the nucleotide sequence determined by sequencing of clone 57-A-2 by using a -21M13 primer wherein the clone 57-A-2 is obtained by amplifying human brain amygdala-derived cDNA by PCR under severe conditions and subcloning it to pCRTM II.

15 Figure 21 shows the nucleotide sequence determined by sequencing of clone B54 with a T7 primer wherein the clone B54 is obtained by amplifying rat whole brain-derived cDNA by PCR under mild conditions and subcloning it to pCRTM II.

20 Figure 22 illustrates the nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in the cDNA clone p19P2 isolated by PCR using a human pituitary gland-derived cDNA and the amino acid sequence encoded thereby, wherein the primer used 25 for sequencing is -21M13, and the underlined part corresponds to the synthetic primer.

Figure 23 illustrates the nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in the cDNA clone p19P2 isolated 30 by PCR using a human pituitary gland-derived cDNA and the amino acid sequence encoded thereby, wherein the primer used for sequencing is M13RV-N (Takara, Japan), and the underlined part corresponds to the synthetic primer.

35 Figure 24 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence

shown in Figure 22.

Figure 25 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence shown in Figure 23.

Figure 26 shows the partial amino acid sequence (p19P2) of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, as shown in Figures 22 and 23, relative to the known G protein coupled receptor protein, S12863, wherein reverse amino acid residues are in agreement, the 1st to 99th amino acid residues of the p19P2 sequence correspond to the 1st to 99th amino acid residues in Figure 22, and the 156th to 230th amino acid residues thereof correspond to the 1st to 68th amino acid residues in Figure 23.

Figure 27 is the nucleotide sequence of the MIN6-derived G protein coupled receptor protein cDNA fragment derived based upon the nucleotide sequences of the MIN6-derived G protein coupled receptor protein cDNA fragments each included in the cDNA clones, pG3-2 and pG1-10, isolated by PCR using a MIN6-derived cDNA and the amino acid sequence encoded thereby, wherein the underlined parts corresponds to the synthetic primers.

Figure 28 is the partial hydrophobicity plotting profile of the MIN6-derived G protein coupled receptor protein, prepared based upon the partial amino acid sequence shown in Figure 27.

Figure 29 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p63A2, obtained from the human amygdaloid nucleus by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined part corresponds to the synthetic primer.

Figure 30 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p63A2, obtained from the human amygdaloid nucleus by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined part

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corresponds to the synthetic primer.

Figure 31 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 29, suggesting the presence of hydrophobic domains as designated by 1 to 3.

Figure 32 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 30, suggesting the presence of hydrophobic domains as designated by 4 to 6.

Figure 33 is the partial amino acid sequence (p63A2) of the protein encoded by the novel receptor protein cDNA fragment included in p63A2, relative to the partial amino acid sequence of the G protein coupled receptor protein (P30731) expressed and induced by a mouse T cell-derived glucocorticoid, wherein reverse amino acid residues are in agreement.

Figure 34 is the whole nucleotide sequence of the the human pituitary gland-derived G protein coupled receptor protein cDNA, included in the cDNA clone, phGR3, isolated from the human-derived cDNA library by plaque hybridization using an DNA insert in the p19P2 as a probe, and the amino acid sequence encoded thereby.

Figure 35 is the northern blotting profile of the human pituitary gland mRNA of the receptor gene encoded by the human pituitary gland-derived cDNA clone, phGR3.

Figure 36 is the hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA included in phGR3, prepared based upon the amino acid sequence shown in Figure 34.

Figure 37 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p3H2-17, obtained from mouse pancreatic β -cell strain, MIN6, by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined part corresponds to the synthetic primer used for the PCR amplification.

Figure 38 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 37, suggesting the presence of hydrophobic domains as designated

by 3 to 6.

Figure 39 is the partial amino acid sequence encoded by the novel receptor protein cDNA included in p3H2-17, relative to the partial amino acid sequence each of chicken ATP receptor protein (P34996), human somatostatin receptor subtype 3 protein (A46226), human somatostatin receptor subtype 4 protein (JN0605) and bovine neuropeptide Y receptor protein (S28787), wherein reverse amino acid residues are in agreement.

Figure 40 is the partial nucleotide sequence of the novel receptor protein cDNA clone, p3H2-34, obtained from mouse pancreatic β -cell strain, MIN6, by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined parts correspond to the synthetic primers used for the PCR amplification.

Figure 41 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 40, wherein the axis of ordinate represents an index of hydrophobicity, the axis of abscissa represents the number of amino acids and numerals 3 to 6 represent the presence of hydrophobic domains.

Figure 42 is the partial amino acid sequence encoded by the novel receptor protein cDNA included in p3H2-34, relative to the partial amino acid sequence each of human somatostatin receptor subtype 4 protein (JN0605), human somatostatin receptor subtype 2 protein (B41795) and rat-derived ligand unknown receptor protein (A39297), wherein reverse amino acid residues are in agreement.

Figure 43 is the nucleotide sequence of the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMD4, obtained from rabbit gastropyloric part smooth muscles by PCR amplification, and the amino acid sequence encoded thereby, wherein the underlined parts correspond to the synthetic primers used for the PCR amplification.

Figure 44 is the hydrophobicity plotting profile of the protein encoded by the rabbit gastropyloric part smooth

muscle-derived G protein coupled receptor protein cDNA fragment included in pMD4, prepared based upon the amino acid sequence shown in Figure 35, wherein numerals 1 to 3 suggest the presence of hydrophobic domains.

5 Figure 45 is the partial amino acid sequence (pMD4) of the protein encoded by the rabbit gastropylolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMD4 as shown in Figure 43, relative to the known G protein coupled receptor protein, rat ligand unknown receptor 10 protein (A35639), wherein reverse amino acid residues are in agreement, the 1st to 88th amino acid residues of the pMD4 sequence correspond to the 1st to 88th amino acid residues in Figure 43.

15 Figure 46 shows the nucleotide sequence of the mouse-derived galanin receptor protein cDNA clone, pMGR20, which has been cloned with, as a probe, the cDNA insert in p3H2-34 and the amino acid sequence encoded thereby.

20 Figure 47 is the hydrophobicity plotting profile, prepared based upon the amino acid sequence shown in Figure 46, wherein the axis of ordinate represents an index of hydrophobic property, the axis of abscissa represents the number of amino acids, and numerals 1 to 7 represent the presence of hydrophobic domains.

25 Figure 48 is the amino acid sequence (MOUSEGALRECE) of the mouse-derived galanin receptor protein encoded by pMGR20, relative to the amino acid sequence (HUMAGALAMI) of the human-derived galanin receptor protein, wherein reverse amino acid residues are in agreement.

30 Figure 49 is the nucleotide sequence of the rabbit gastropylolic part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMJ10, obtained from rabbit gastropylolic part smooth muscles by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined parts 35 corresponds to the synthetic primers used for the PCR amplification.

Figure 50 is the hydrophobicity plotting profile of

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the protein encoded by the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDBA fragment included in pMJ10, prepared based upon the amino acid sequence shown in Figure 49, wherein numerals 4 to 6 suggest the
5 presence of hydrophobic domains.

Figure 51 is the partial amino acid sequence (pMJ10) of the protein encoded by the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMJ10 shown in Figure 49, relative to human ligand
10 unknown receptor protein (B42009), human N-formylpeptide receptor protein (JC2014), rabbit N-formylpeptide receptor protein (A46520), mouse C5a anaphylatoxin receptor protein (A46525) and bovine neuropeptide Y receptor protein (S28787) which are known G protein coupled receptor proteins, wherein
15 reverse amino acid residues are in agreement, and the 1st to 125th amino acid residues of pMJ10 correspond to the 1st to 125th amino acid residues in Figure 49.

Figure 52 is the nucleotide sequence of the rabbit gastropyloric part smooth muscle-derived G protein coupled
20 receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMH28, obtained from rabbit gastropyloric part smooth muscles by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined parts correspond to the synthetic primers used for the PCR
25 amplification.

Figure 53 is the hydrophobicity plotting profile of the protein encoded by the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDBA fragment included in pMH28, prepared based upon the amino acid sequence shown in Figure 52, wherein numerals 4 to 6 suggest the
30 presence of hydrophobic domains.

Figure 54 is the partial amino acid sequence (pMH28) of the protein encoded by the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMH28 shown in Figure 52, relative to mouse IL-8 receptor protein (P35343), human somatostatin receptor protein 1 (A41795) and human somatostatin receptor protein 4 (A47457)

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which are known G protein coupled receptor proteins, wherein reverse amino acid residues are in agreement, and the 1st to 119th amino acid residues of pMH28 correspond to the 1st to 119th amino acid residues in Figure 52.

5 Figure 55 is the nucleotide sequence of the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMN7, obtained from rabbit gastropyloric part smooth muscles by PCR amplification and the amino acid 10 sequence encoded thereby, wherein the underlined 5'-end nucleotide sequence part corresponds to the synthetic primer used for the PCR amplification.

15 Figure 56 is the nucleotide sequence of the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMN7, obtained from rabbit gastropyloric part smooth muscles by PCR amplification and the amino acid sequence encoded thereby, wherein the underlined 3'-end nucleotide sequence part corresponds to the synthetic primer 20 used for the PCR amplification.

25 Figure 57 is the hydrophobicity plotting profile of the protein encoded by the rabbit gastropyloric part smooth muscle- derived G protein coupled receptor protein cDNA fragment included in pMN7, prepared based upon the amino acid sequences shown in Figures 55 and 56, wherein numerals TM2 to TM6 suggest the presence of hydrophobic domains.

30 Figure 58 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence shown in Figure 22.

35 Figure 59 is the partial hydrophobicity plotting profile of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, prepared based upon the amino acid sequence shown in Figure 23.

Figure 60 shows the partial amino acid sequence

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(p19P2) of the protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2, as shown in Figures 22 and 23, relative to the known G protein coupled receptor protein, S12863, wherein
5 reverse amino acid residues are in agreement, the 1st to 99th amino acid residues of the p19P2 sequence correspond to the 1st to 99th amino acid residues in Figure 22, and the 156th to 230th amino acid residues thereof correspond to the 1st to 68th amino acid residues in Figure 23.

10 Figure 61 is the partial amino acid sequence (pG3-2/pG1-10) of the MIN6-derived G protein coupled receptor protein, as shown in Figure 27, relative to the partial amino acid sequence (p19P2) of the protein encoded by p19P2, as shown in Figures 22 and 23, wherein reverse amino acid residues are
15 in agreement, the 1st to 99th amino acid residues of the p19P2 sequence correspond to the 1st to 99th amino acid residues in Figure 22, the 156th to 223rd amino acid residues thereof correspond to the 1st to 68th amino acid residues in Figure 23, and the 1st to 223rd amino acid residues of the
20 pG3-2/pG1-10 sequence correspond to the 1st to 223rd amino acid residues in Figure 27.

Figure 62 is the nucleotide sequence of the MIN6-derived G protein coupled receptor protein cDNA fragment included in the cDNA clone, p5S38, isolated by PCR using a
25 MIN6-derived cDNA and the amino acid sequence encoded thereby, wherein the underlined parts corresponds to the synthetic primers.

30 Figure 63 is the partial amino acid sequence (p5S38) of the MIN6-derived G protein coupled receptor protein, as shown in Figure 62, relative to the partial amino acid sequence (p19P2) of the G protein coupled receptor protein encoded by p19P2, as shown in Figures 22 and 23, as well as the partial amino acid sequence of the G protein coupled receptor protein encoded by the nucleotide sequence derived from the nucleotide sequence of the cDNA fragment included in pG3-2 and pG1-10, as
35 shown in Figure 27, wherein reverse amino acid residues are in agreement, the 1st to 144th amino acid residues of the p5S38

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sequence correspond to the 1st to 144th amino acid residues in Figure 62, the 1st to 99th amino acid residues of the p19P2 sequence correspond to the 1st to 99th amino acid residues in Figure 22, the 156th to 223rd amino acid residues thereof 5 correspond to the 1st to 68th amino acid residues in Figure 23, and the 1st to 223rd amino acid residues of the pG3-2/pG1-10 sequence correspond to the 1st to 223rd amino acid residues in Figure 27.

Figure 64 is the partial hydrophobicity plotting 10 profile of the protein encoded by the MIN6-derived G protein coupled receptor protein cDNA fragment included in p5S38, prepared based upon the amino acid sequence shown in Figure 62.

Figure 65 shows the northern blot analysis profile 15 of the receptor gene encoded by the cDNA included in the mouse pancreatic β -cell strain MIN6-derived novel receptor protein cDNA clone, p3H2-17, for mouse cell line, MIN6, Neuro-2a cell and mouse brain, thymus, spleen and pancreas poly(A)⁺ RNA, wherein each arrow and number indicates the size marker position (unit of number: kb).

Figure 66 shows the agarose gel electrophoresis 20 analysis profile of the PCR products obtained by 5'RACE PCR of the receptor gene included in p3H2-17 using mouse thymus and spleen poly(A)⁺ RNA.

Lane 1 indicates the size marker 6 (Wako Pure 25 Chemical, Japan).

Lane 2 indicates the internal control which is the thymus-derived PCR product obtained by PCR amplification using the primer having SEQ ID NO: 20 and the primer having SEQ ID NO: 22 with Taq polymerase.

Lane 3 indicates the negative control which is the 30 PCR product obtained by Ex Tag polymerase PCR amplification of thymus cDNA prior to addition of anchors.

Lane 4 indicates the negative control which is the 35 PCR product obtained by Tag polymerase PCR amplification of thymus cDNA prior to addition of anchors.

Lane 5 indicates the PCR product obtained by 5'RACE of thymus poly(A)⁺ RNA with Pfu polymerase.

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Lane 6 indicates the PCR product obtained by 5'RACE of thymus poly(A)⁺ RNA with Vent polymerase.

Lane 7 indicates the PCR product obtained by 5'RACE of thymus poly(A)⁺ RNA with Ex Tag polymerase.

5 Lane 8 indicates the PCR product obtained by 5'RACE of thymus poly(A)⁺ RNA with Taq polymerase.

Lane 9 indicates the size marker 5 (Wako Pure Chemical, Japan).

10 Lane 10 indicates the internal control which is the spleen-derived PCR product obtained by PCR amplification using the primer having SEQ ID NO: 20 and the primer having SEQ ID NO: 22 with Taq polymerase.

15 Lane 11 indicates the negative control which is the PCR product obtained by Ex Tag polymerase PCR amplification of spleen cDNA prior to addition of anchors.

Lane 12 indicates the negative control which is the PCR product obtained by Tag polymerase PCR amplification of spleen cDNA prior to addition of anchors.

20 Lane 13 indicates the PCR product obtained by 5'RACE of poly(A)⁺ RNA with Pfu polymerase.

Lane 14 indicates the PCR product obtained by 5'RACE of spleen poly(A)⁺ RNA with Vent polymerase.

Lane 15 indicates the PCR product obtained by 5'RACE of spleen poly(A)⁺ RNA with Ex Tag polymerase.

25 Lane 16 indicates the PCR product obtained by 5'RACE of spleen poly(A)⁺ RNA with Tag polymerase.

Lane 17 indicates the size marker 5 (Wako Pure Chemical, Japan).

30 Each blacked triangle indicates the band recovered.

Figure 67 shows the agarose gel electrophoresis analysis profile of the PCR products obtained by 3'RACE PCR of the receptor gene included in p3H2-17 using mouse thymus and spleen poly(A)⁺ RNA.

35 Lane 1 indicates the size marker 5 (Wako Pure Chemical, Japan).

Lane 2 indicates the PCR product obtained by 3'RACE of spleen poly(A)⁺ RNA with Tag polymerase.

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Lane 3 indicates the PCR product obtained by 3'RACE of spleen poly(A)⁺ RNA with Ex Taq polymerase.

Lane 4 indicates the PCR product obtained by 3'RACE of spleen poly(A)⁺ RNA with Vent polymerase.

5 Lane 5 indicates the PCR product obtained by 3'RACE of spleen poly(A)⁺ RNA with Pfu polymerase.

Lane 6 indicates the PCR product obtained by 3'RACE of thymus poly(A)⁺ RNA with Taq polymerase.

10 Lane 7 indicates the PCR product obtained by 3'RACE of thymus poly(A)⁺ RNA with Ex Taq polymerase.

Lane 8 indicates the PCR product obtained by 3'RACE of thymus poly(A)⁺ RNA with Vent polymerase.

Lane 9 indicates the PCR product obtained by 3'RACE of thymus poly(A)⁺ RNA with Pfu polymerase.

15 Lane 10 indicates the size marker 6 (Wako Pure Chemical, Japan).

Each blacked triangle indicates the band recovered.

Figure 68 depicts the model of the RACE products of the receptor protein cDNA fragment included in p3H2-17 obtained by 5'RACE and 3'RACE. Open squares represent regions which have already been isolated and included in p3H2-17. Small arrows, ①, ②, ③ and ④, indicate the positions and directions of the primers designed in Working Example 19. The big arrow shows a predicted full-length open reading frame of the receptor protein held by p3H2-17. Numbers at both ends, N26, N64, N75, C2, C13 and C15, indicate clone numbers of the RACE products obtained. Among these RACE products, N26, N64 and N75 are inserted into pPCRTM II vector and C2, C13 and C15 are inserted into the SmaI site of pUC18. The solid triangle indicates the PCR error position which has been clarified through sequencing.

35 Figure 69 is the nucleotide sequence of the open reading frame and neighboring regions thereof of mouse G protein coupled receptor protein cDNA included in the cDNA clone pMAH2-17 obtained from mouse spleen and thymus poly(A) RNA by RACE techniques based on the nucleotide sequence of the cDNA fragment included in p3H2-17 and the amino acid

sequence encoded thereby.

Figure 70 is the hydrophobicity plotting profile of the protein encoded by the receptor protein cDNA included in pMAH2-17, prepared based upon the amino acid sequence shown in Figure 69.

Figure 71 is the amino acid sequence (75+13CODING) of the protein encoded by the mouse-derived G protein coupled receptor protein cDNA fragment included in pMAH2-17, as shown in Figure 69, relative to the known G protein coupled receptor proteins, mouse P_{2U}purinoceptor (P2UR MOUSE) and chicken P_{2Y} purinoceptor (P2YR CHICK), wherein reverse amino acid residues are in agreement.

Figure 72 is the nucleotide sequence (from 1st to 540th nucleotides) of the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMN128, obtained from rabbit gastropyloric part smooth muscles by PCR amplification, and the amino acid sequence encoded thereby, wherein the underlined 5' part corresponds to the synthetic primer used for the PCR amplification.

Figure 73 is the nucleotide sequence (from 541st to 843rd nucleotides) of the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in the novel receptor protein cDNA clone, pMN128, obtained from rabbit gastropyloric part smooth muscles by PCR amplification, and the amino acid sequence encoded thereby, wherein the underlined 3' part corresponds to the synthetic primer used for the PCR amplification.

Figure 74 is the hydrophobicity plotting profile of the protein encoded by the rabbit gastropyloric part smooth muscle-derived G protein coupled receptor protein cDNA fragment included in pMN128, prepared based upon the amino acid sequences shown in Figures 72 and 73, suggesting the presence of hydrophobic domains.

Figure 75 shows inward currents evoked by ATP in Xenopus oocytes injected with cDNA of pMAH2-17-encoded receptor.

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Figure 76 is the nucleotide sequence of the human-derived G protein coupled receptor protein cDNA fragment included in pH3H2-17, relative to the nucleotide sequence of the mouse-derived G protein coupled receptor protein cDNA fragment included in p3H2-17, wherein reverse base residues are in agreement.

Figure 77 is the nucleotide sequence of the open reading frame and neighboring regions thereof of human-derived G protein coupled receptor protein cDNA included in pHAH2-17 and the amino acid sequence encoded thereby.

Figure 78 is the hydrophobicity plotting profile of the protein encoded by the human-derived G protein coupled receptor protein cDNA included in pHAH2-17.

Figure 79 is the amino acid sequence of human type purinoceptor encoded by pHAH2-17, relative to the mouse purinoceptor encoded by p3H2-17, wherein reverse amino acid residues are in agreement.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

According to the present invention, DNA sequences comprising each a nucleotide sequence indicated by a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 19 have been synthesized and characterized. The DNA is a potent primer for polymerase chain reaction in order to amplify DNA sequences encoding part or all of the polypeptide sequence of G protein coupled receptor protein. PCR amplification methods of the DNA coding for part or all of the polypeptide sequence of G protein coupled receptor protein can be advantageously carried out with the said primer DNA.

Screening of DNA libraries for the DNA encoding part or all of the polypeptide sequence of G protein coupled receptor protein can be successfully carried out through polymerase chain reaction techniques with the said primer DNA.

As a result, template DNAs coding for part or all of the polypeptide sequence of G protein coupled receptor protein, contained in the DNA library, can be selectively amplified and various DNA sequences encoding part or all of the

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polypeptide sequence of G protein coupled receptor protein may be isolated and characterized. Further, G protein coupled receptor proteins, peptide segments or fragments derived from the G protein coupled receptor protein, modified derivatives or 5 analogues thereof, and salts thereof may be recognized, predicted, deduced, produced, expressed, isolated and characterized.

The primer DNA useful in PCR amplification of the DNA sequence encoding part or all of the polypeptide sequence 10 of G protein coupled receptor protein is a degenerate deoxynucleotide which has an oligonucleotide sequence to which a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 19 is assigned.

15 The nucleotide sequence represented by SEQ ID NO: 1 is a base sequence having the following formula:

5'-CGTGGSCMTSSTGGGCAACN₁YCCTG-3'

wherein S is G or C, M is A or C, N₁ = A, G, C, or T, and Y is T or C (Figure 1: HS-1).

20 The nucleotide sequence represented by SEQ ID NO: 2 (HS-2) is a base sequence having the following formula:

5'-GTN₁GWRRGGCAN₁CCAGCAGAKGGCAAA-3'

wherein N₁ = A, G, C, or T, W is A or T, R is A or G, and K is G or T, which is complementary to a nucleotide sequence having the following formula:

25 5'-TTGCCMTCTGCTGGNTGCCYYWCNAC-3'

wherein N = A, C, G, or T, M is A or C, Y is T or C, and W is A or T (Figure 2).

30 The nucleotide sequence represented by SEQ ID NO: 3 is a base sequence having the following formula:

5'-CTCGCSGCYMTN₂RGYATGGAYCGN₂TAT-3'

wherein S is G or C, Y is C or T, M is A or C, R is A or G, and N₂ = I (Figure 4: 3D).

35 The nucleotide sequence represented by SEQ ID NO: 4 is a base sequence having the following formula:

5'-CATGTRGWAGGGAAN₂CCAGSAMAN₂RARRAA-3'

wherein R is A or G, W is T or A, S is G or C, M is A or C, and N₂ = I, which is complementary to a nucleotide sequence

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having the following formula:



wherein Y is C or T, N₁ = A, G; C, or T, K is G or T, S is G or C, W is A or T (Figure 6: 6C).

5 The nucleotide sequence represented by SEQ ID NO: 5
is a base sequence having the following formula:



wherein Y is C or T, R is A or G, S is G or C, M is A or C, and V is A, C or G, and N₂ is I (Figure 3: 3A).

10 The nucleotide sequence represented by SEQ ID NO: 6
is a base sequence having the following formula:



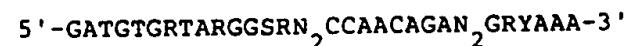
wherein Y is C or T, R is A or G, S is G or C, M is A or C, and V is A, C or G, and N₂ is I (Figure 3: 3B).

15 The nucleotide sequence represented by SEQ ID NO: 7
is a base sequence having the following formula:



wherein S is G or C, Y is C or T, M is A or C, R is A or G, and N₂ is I (Figure 4: 3C).

20 The nucleotide sequence represented by SEQ ID NO: 8
is a base sequence having the following formula:

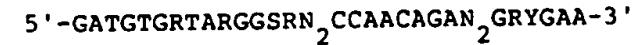


wherein R is A or G, S is G or C, Y is C or T, and N₂ is I,
which is complementary to a nucleotide sequence having the
following formula:



wherein R is A or G, Y is C or T, S is G or C, and N₁ is A,
T, G, or C (Figure 5: 6A).

25 The nucleotide sequence represented by SEQ ID NO: 9
is a base sequence having the following formula:



wherein R is A or G, S is G or C, Y is C or T, and N₂ is I,
which is complementary to a nucleotide sequence having the
following formula:



35 wherein R is A or G, Y is C or T, S is G or C, and N₁ is A,

- 7 9 -

T, G, or C (Figure 5: 6B).

The nucleotide sequence represented by SEQ ID NO: 10 is a base sequence having the following formula:

5'-GYCACCAACN₂WSTTCATCCTSWN₂HCTG-3'

5 wherein S is G or C, Y is C or T, W is A or T, H is A, C or T, and N₂ is I (Figure 7: T2A).

The nucleotide sequence represented by SEQ ID NO: 11 (Figure 8: T7A) is a base sequence having the following formula:

10 5'-ASN₂SAN₂RAAGSARTAGAN₂GAN₂RGGRTT-3'

wherein R is A or G, S is G or C, and N₂ is I, which is complementary to a nucleotide sequence having the following formula:

5'-AAYCCYN₂TCN₂TCTAYTSCTTYN₂TSN₂ST-3'

15 wherein Y is C or T, N₂ is I, and S is G or C (Figure 8).

The nucleotide sequence represented by SEQ ID NO: 12 is a base sequence having the following formula:

5'-TGN₂TSSTKMTN₂GSN₂GKGTN₂GGN₂AA-3'

wherein S is G or C, K is G or T, M is A or C, and N₂ is I
20 (Figure 9: TM1-A2).

The nucleotide sequence represented by SEQ ID NO: 13 (Figure 10: TM3-B2) is a base sequence having the following formula:

5'-AYCKGTAYCKGTCCAN₂KGWN₂ATKGC-3'

25 wherein Y is C or T, K is G or T, W is A or T, and N₂ is I, which is complementary to a nucleotide sequence having the following formula:

5'-GCMATN₂WCMN₂TGGACMGRTACMGRT-3'

wherein M is A or C, W is A or T, R is A or G, and N₂ is I
30 (Figure 10).

The nucleotide sequence represented by SEQ ID NO: 14 is a base sequence having the following formula:

5'-CATKKCCSTGGASAGN₂TAYN₂TRGC-3'

wherein K is G or T, S is G or C, Y is C or T, R is A or G, and N₂ is I (Figure 11: TM3-C2).

The nucleotide sequence represented by SEQ ID NO: 15 (Figure 12: TM6-E2) is a base sequence having the following

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formula:

5'-GWWGGGSAKCCAGCASA₂N₂GGCRAA-3'

wherein W is A or T, S is G or C, K is G or T, R is A or G, and N₂ is I, which is complementary to a nucleotide sequence

5 having the following formula:

5'-TTYGCCN₂TSTGCTGGMTSCCCWWC-3'

wherein Y is C or T, S is G or C, M is A or C, W is A or T, and N₂ is I (Figure 12).

10 The nucleotide sequence represented by SEQ ID NO: 16 is a base sequence having the following formula:

5'-ARYYTN₂GCN₂N₂TN₂GCN₁YAY-3'

wherein R is A or G, Y is C or T, N₁ is A, T, G, or C, and N₂ is I (Figure 13: TM2F18).

15 The nucleotide sequence represented by SEQ ID NO: 17 (Figure 14: TM6R21) is a base sequence having the following formula:

5'-N₂GGN₂AN₂CCARCAN₁AN₁N₁RN₁RAA-3'

wherein R is A or G, N₁ is A, T, G, or C, and N₂ is I

which is complementary to a nucleotide sequence having the 20 following formula:

5'-TTYN₁YN₁N₁TN₁TGYTGGN₂TN₂CCN-3'

wherein Y is C or T, N₁ is A, T, G, or C, and N₂ is I

(Figure 14).

25 The nucleotide sequence represented by SEQ ID NO: 18 is a base sequence having the following formula:

5'-GCCTSN₂TN₂RN₂SATGWSTGTGGAN₂MGN₂T-3'

wherein S is G or C, R is A or G, W is A or T, M is A or C, and N₂ is I (Figure 15: S3A).

30 The nucleotide sequence represented by SEQ ID NO: 19 (Figure 16: S6A) is a base sequence having the following formula:

5'-GAWSN₂TGMYN₂AN₂RTGGWAGGGN₂AN₂CCA-3'

wherein W is A or T, S is G or C, M is A or C, Y is C or T, R is A or G, and N₂ is I, which is complementary to a 35 nucleotide sequence having the following formula:

5'-TGGN₂TN₂CCCTWCCAYN₂TN₂RKCAN₂SWTC-3'

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wherein W is A or T, Y is C or T, R is A or G, K is G or T, and S is G or C (Figure 16).

In a specific embodiment, symbols in the aforementioned SEQ ID NOS (R, Y, M, K, S, W, H, V and N) 5 indicate the incorporation of plural bases, leading to multiple oligonucleotides in the primer preparation. In other words, SEQ ID NO: 1 to SEQ ID NO: 19 are degenerate nucleotide primers.

The nucleotide sequence represented by SEQ ID NO: 1 (Figure 1: HS-1) is a nucleotide sequence highly homologous to 10 the DNA sequence coding for the amino acid sequence corresponding to or near the first membrane-spanning (transmembrane) domain each of known G protein coupled receptor proteins such as human-derived TRH receptor protein (HTRHR), human-derived RANTES receptor protein (L10918, HUMRANTES), 15 human Burkitt's lymphoma-derived receptor protein with an unknown ligand (X68149, HSBLR1A), human-derived somatostatin receptor protein (L14856, HUMSOMAT0), rat-derived μ -opioid receptor protein (U02083, RNU02083), rat-derived κ -opioid receptor protein (U00442, U00442), human-derived 20 neuromedin B receptor protein (M73482, HUMNMBR), human-derived muscarinic acetylcholine receptor protein (X15266, HSHM4), rat-derived adrenaline α_1 B receptor protein (L08609, RATAADRE01), human-derived somatostatin 3 receptor protein (M96738, HUMSSTR3X), 25 human-derived C_5 a receptor protein (HUMC5AAR), human-derived receptor protein with an unknown ligand (HUMRDC1A), human-derived receptor protein with an unknown ligand (M84605, HUMOPIODRE), rat-derived adrenaline α_2 B receptor protein (M91466, RATA2BAR) and the like 30 [Figure 1].

The nucleotide sequence represented by SEQ ID NO: 2 (HS-2) is a nucleotide sequence which is complementary to the nucleotide sequence (Figure 2) highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or 35 near the sixth membrane-spanning domain of known G protein coupled receptor proteins such as mouse-derived receptor

- 8 2 -

protein with an unknown ligand (M80481, MUSGIR), human-derived bombesin receptor protein (L08893, HUMBOMB3S), human-derived adenosine A2 receptor protein (S46950, S46950), mouse-derived receptor protein with an unknown ligand (D21061, MUSGPCR),
5 mouse-derived TRH receptor protein (S43387, S43387), rat-derived neuromedin K receptor protein (J05189, RATNEURA), rat-derived adenosine A1 receptor protein (M69045, RATA1ARA), human-derived neurokinin A receptor protein (M57414, HUMNEKAR), rat-derived adenosine A3 receptor protein (M94152, RATADENREC),
10 human-derived somatostatin 1 receptor protein (M81829, HUMSTR1A), human-derived neurokinin 3 receptor protein (S86390, S86371S4), rat-derived receptor protein with an unknown ligand (X61496, RNCGPCR), human-derived somatostatin 4 receptor protein (L07061, HUMSSTR4Z), rat-derived GnRH
15 receptor protein (M31670, RATGNRHA) and the like [Figure 2].

The nucleotide sequence represented by SEQ ID NO: 5 (Figure 3: 3A) or the nucleotide sequence represented by SEQ ID NO: 6 (Figure 3: 3B) is a nucleotide sequence highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the third membrane-spanning domain each of known G protein coupled receptors such as mouse-derived α -opioid receptor protein (L11064), mouse-derived δ -opioid receptor protein (L11065), rat-derived μ -opioid receptor protein (D16349), mouse-derived bradykinin B2 receptor protein (X69676), rat-derived bradykinin B2 receptor protein (M59967), mouse-derived bombesin receptor protein (M35328), human-derived neuromedin B receptor protein (M73482), human-derived gastrin releasing peptide receptor protein (M73481), human-derived bombesin receptor protein subtype 3 (L08893), mouse-derived substance K receptor protein (X62933), mouse-derived substance P receptor protein (X62934), rat-derived neurokinin 3 receptor protein (J05189), rat-derived endothelin receptor protein (M60786), rat-derived receptor protein with an unknown ligand (L04672), rat-derived receptor protein with an unknown ligand (X61496), rat-derived receptor protein with an unknown ligand (X59249), rat-derived receptor protein with an unknown ligand (L09249),
20
25
30
35

- 8 3 -

mouse-derived receptor protein with an unknown ligand (P30731), human-derived receptor protein with an unknown ligand (M31210), human-derived receptor protein with an unknown ligand (U03642) and the like [Figure 3].

5 The nucleotide sequence represented by SEQ ID NO: 7 (Figure 4: 3C) or the nucleotide sequence represented by SEQ ID NO: 3 (Figure 4: 3D) is a nucleotide sequence highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the third membrane-spanning domain each of known G protein coupled receptors such as mouse-derived angiotensin II receptor protein (L32840), rat-derived angiotensin Ib receptor protein (X64052), rat-derived angiotensin receptor protein subtype (M90065), human-derived angiotensin Ia receptor protein (M91464),
10 rat-derived cholecystokinin a receptor protein (M88096), rat-derived cholecystokinin b receptor protein (M99418), human-derived cholecystokinin b receptor protein (L04473), mouse-derived low affinity interleukin 8 receptor protein (M73969), human-derived high affinity interleukin 8 receptor
15 protein (X65858), mouse-derived C5a anaphylatoxin receptor protein (S46665), human-derived N-formylpeptide receptor protein (M60626) and the like [Figure 4].

20 The nucleotide sequence represented by SEQ ID NO: 10 (Figure 7: T2A) is a nucleotide sequence highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the second membrane-spanning domain each of known G protein coupled receptors such as human galanin receptor (HUMGALAREC), rat α -1B-adrenergic receptor (RATADR1B), human β -1-adrenergic receptor (HUMADRB1), rabbit IL-8 receptor (RABIL8RSB), human opioid receptor (HUMOPIODRE), bovine substance K receptor (BTSKR), human somatostatin receptor-2 (HUMSRI2A), human somatostatin receptor-3 (HUMSSTR3Y), human gastrin receptor (HUMGARE), human cholecystokinin A receptor (HUMCCKAR), human dopamine receptor-D5 (HUMD1B), human serotonin receptor 5HT1E (HUM5HT1E), human dopamine receptor D4 (HUMD4C), mouse serotonin receptor-2 (MMSERO), rat α -1A-adrenergic receptor
25 30 35

- 8 4 -

(RATADRA1A), rat histamine H₂ receptor (S57565) and the like [Figure 7].

The nucleotide sequence represented by SEQ ID NO: 8 (complementary to 6A of Figure 5) or the nucleotide sequence 5 represented by SEQ ID NO: 9 (complementary to 6B of Figure 5) is a nucleotide sequence which is complementary to the nucleotide sequence (Figure 5) highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the sixth membrane-spanning domain of known G protein coupled receptors such as mouse-derived κ -opioid 10 receptor protein (L11064), mouse-derived δ -opioid receptor protein (L11065), rat-derived μ -opioid receptor protein (D16349), mouse-derived bradykinin B₂ receptor protein (X69676), rat-derived bradykinin B₂ receptor protein (M59967), 15 mouse-derived bombesin receptor protein (M35328), human-derived neuromedin B receptor protein (M73482), human-derived gastrin releasing peptide receptor protein (M73481), human-derived bombesin receptor protein subtype 3 (L08893), mouse-derived substance K receptor protein (X62933), 20 mouse-derived substance P receptor protein (X62934), rat-derived neurokinin 3 receptor protein (J05189), rat-derived endothelin receptor protein (M60786), rat-derived receptor protein with an unknown ligand (L04672), rat-derived receptor protein with an unknown ligand (X61496), 25 rat-derived receptor protein with an unknown ligand (X59249), rat-derived receptor protein with an unknown ligand (L09249), mouse-derived receptor protein with an unknown ligand (P30731), human-derived receptor protein with an unknown ligand (M31210) human-derived receptor protein with an unknown ligand (U03642) 30 and the like [Figure 5].

The nucleotide sequence represented by SEQ ID NO: 4 (complementary to 6C of Figure 6) is a nucleotide sequence which is complementary to the nucleotide sequence (Figure 6) highly homologous to the DNA sequence coding for the amino acid 35 sequence corresponding to or near the sixth membrane-spanning domain of known G protein coupled receptors such as mouse-derived angiotensin II receptor protein (L32840),

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rat-derived angiotensin Ib receptor protein (X64052),
rat-derived angiotensin receptor protein subtype (M90065),
human-derived angiotensin Ia receptor protein (M91464),
rat-derived cholecystokinin a receptor protein (M88096),
5 rat-derived cholecystokinin b receptor protein (M99418),
human-derived cholecystokinin 8 receptor protein (L04473),
mouse-derived low affinity interleukin 8 receptor protein
(M73969), human-derived high affinity interleukin 8 receptor
protein (X65858), mouse-derived C5a anaphylatoxin receptor
10 protein (S46665), human-derived N-formylpeptide receptor
protein (M60626) and the like [Figure 6].

The nucleotide sequence represented by SEQ ID NO: 11
(Figure 8: T7A) is a nucleotide sequence which is
complementary to the nucleotide sequence (Figure 8) highly
15 homologous to the DNA sequence coding for the amino acid
sequence corresponding to or near the seventh
membrane-spanning domain each of known G protein coupled
receptors such as human galanin receptor (HUMGALAREC),
rat Al adenosine receptor (RAT1DREC), porcine angiotensin
20 receptor (PIGA2R), rat serotonin receptor (RAT5HTRTC),
human dopamine receptor (S58541), human gastrin releasing
peptide receptor (HUMGRPR), mouse GRP/bombesin receptor
(MUSGRPBM), rat vascular type 1 angiotensin receptor
(RRVT1AIIR), human muscarinic acetylcholine receptor (HSHM4),
25 human β -1 adrenergic receptor (HUMDRB1), human gastrin
receptor (HUMGARE), rat cholecystokinin receptor (RATCCKAR),
rat receptor with an unknown ligand (S59748), human
somatostatin receptor (HUMSST28A), rat receptor with an unknown
ligand (RNGPROCR), mouse somatostatin receptor-1 (MUSSRI1A),
30 human α -Al-adrenergic receptor (HUMA1AADR), mouse
delta-opioid receptor (S66181), human somatostatin receptor-3
(HUMSSTR3Y) and the like [Figure 8].

The nucleotide sequence represented by SEQ ID NO: 12
(Figure 9: TM1-A2) is a nucleotide sequence highly homologous
35 to the DNA sequence coding for the amino acid sequence
within the first membrane-spanning (transmembrane) domain
each of known G protein coupled receptors such as

mouse-derived bradykinin B₂ receptor (MUSBB2R),
bovine-derived substance K receptor (BTSKR), bovine-derived
endothelin ET_B receptor (BOVEETBR), human-derived
neuropeptide Y receptor (MMSUBKREC), human-derived
5 prostaglandin E₂ receptor (HUMPGE2R), human-derived
prostacyclin receptor (HUMPIR), human-derived κ -opioid
receptor (HSU11053), rat-derived melanocortin 3 receptor
(RRMC3RA), human-derived melanocortin receptor (HUMMR),
mouse-derived bombesin/GRP receptor (MUSGRPBM),
10 rat-derived cholecystokinin B receptor (RATCHOLREC),
rat-derived cholecystokinin A receptor (RATCCKAR) and the
like [Figure 9].

The nucleotide sequence represented by SEQ ID NO: 13
(Figure 10: TM3-B2) is a nucleotide sequence which
15 is complementary to the nucleotide sequence (Figure 10) highly
homologous to the DNA sequence coding for the amino acid
sequence corresponding to or near the end of the third
membrane-spanning domain of known G protein coupled receptors
such as human-derived cholecystokinin receptor (HUMCCKR),
20 human-derived cholecystokinin B receptor (HUMCCKBGR),
mouse-derived melanocortin 5 receptor (MMGMC5R),
human-derived vasopressin receptor (HUMV2R), rat-derived
neuromedin K receptor (RATNEURA), dog-derived gastrin receptor
(DOGGSTRN), rat-derived serotonin receptor (RAT5HT5A),
25 mouse-derived α_2 -adrenalin receptor (MUSALP2ADA),
human-derived adenosine A₁ receptor (HUMADORA1X),
human-derived opioid (presumed) receptor (HUMOPIOGRE),
mouse-derived bombesin/GRP receptor (MUSGRPBM),
rat-derived cholecystokinin A receptor (RATCCKAR),
30 human-derived TRH receptor (HSTRHREC) and the like [Figure 10].

The nucleotide sequence represented by SEQ ID NO: 14
(Figure 11: TM3-C2) is a nucleotide sequence highly homologous
to the DNA sequence coding for the amino acid sequence
corresponding to or near the end of the third membrane-spanning
35 domain of known G protein coupled receptors such as
human-derived neurokinin 3 receptor (HUMNK3R), human-derived
oxytocin receptor (HSMRNAOXY), guinea pig-derived

cholecystokinin A receptor (S68242), dog-derived
cholecystokinin A receptor with an unknown ligand (CFGPCR4),
mouse-derived substance P receptor (MMSUBPREC), human-derived
receptor with an unknown ligand (HUMOPIODRE), human-derived
5 galanin receptor (HUMGALAREC), human-derived serotonin
receptor (HSS31G), human-derived β_3 -adrenaline receptor
(HUMARB3A), human-derived prostacyclin receptor (HUMHPR),
rat-derived cholecystokinin A receptor (RATCCKAR) and the
like [Figure 11].

10 The nucleotide sequence represented by SEQ ID NO: 15
(Figure 12: TM6-E2) is a nucleotide sequence which
is complementary to the nucleotide sequence (Figure 12) highly
homologous to the DNA sequence coding for the amino acid
sequence within the sixth membrane-spanning domain of known
15 G protein coupled receptors such as human-derived neurokinin A
receptor (HUMNEKAR), human-derived substance P receptor
(HUMSUBPRA), rat-derived substance K receptor (RATSKR),
mouse-derived bombesin/GRP receptor (MUSGRPBM),
human-derived opioid (presumed) receptor (HUMOPIODRE),
20 human-derived adenosine A₂ receptor (HUMA2XXX),
human-derived β_2 -adrenaline receptor (HUMADRBR),
canine-derived receptor RDC5 with an unknown ligand (CFGPCR8),
human-derived endothelin receptor (HUMETSR), mouse-derived
neuropeptide Y1 receptor (MMNPY1CDS), human-derived oxytocin
25 receptor (HSMRNAOXY), rat-derived cholecystokinin A receptor
(RATCCKAR) and the like [Figure 12].

The nucleotide sequence represented by SEQ ID NO: 16
(Figure 13: TM2F18) is a nucleotide sequence highly homologous
to the DNA sequence coding for the amino acid sequence
30 corresponding to or near the second membrane-spanning domain
of known G protein coupled receptors such as human-derived TSH
receptor (HUMTSHX), human-derived neurokinin A receptor
(HUMNEKAR), human-derived FMLP receptor (HUMFMLP),
human-derived IL8 receptor B (HUMINTLEU8), human-derived
35 α -A1 adrenergic receptor (HUMA1AADR), human-derived IL8
receptor A (HUMIL8RA), human-derived dopamine D2 receptor
(HSDD2), human-derived angiotensin type I receptor (HUMANTIR),

human-derived somatostatin receptor (HUSOMAT), human-derived TRH receptor (HSTRHREC), human-derived delta-opioid receptor (HSUO7882) and the like [Figure 13].

The nucleotide sequence represented by SEQ ID NO: 17
5 (Figure 14: TM6R21) is a nucleotide sequence which is
complementary to the nucleotide sequence (Figure 14) highly
homologous to the DNA sequence coding for the amino acid
sequence corresponding to or near the sixth membrane-spanning
domain of known G protein coupled receptors such as
10 human-derived β -adrenergic receptor (HSBAR), human-derived
neurokinin A receptor (HUMNEKAR), human-derived endothelin-1
receptor (HUMETN1R), human-derived histamine H₂ receptor
(HUMHISH2R), human-derived α -A1 adrenergic receptor
(HUMA1AADR), human-derived IL8 receptor A (HUMIL8RA),
15 human-derived neuromedin B receptor (HUMNMBR), human-derived
neurokinin 1 receptor (HUMNKIRX), human-derived substance P
receptor (HUMSUBPRA), human-derived 5-HT1D serotonin receptor
(HUM5HT1DA), human-derived formylpeptide receptor (HUMPPR2A),
human-derived dopamine D2 receptor (HSDD2), human-derived
20 neuropeptide Y receptor (HUMNEYREC), human-derived adenosine
A2 receptor (HUMA2XXX), human-derived bradykinin receptor BK-2
(HUMBK2A), human-derived FMLP-related receptor II (HUMFMLPX),
human-derived somatostatin receptor subtype 3 (HUMSSTR3X),
human-derived cholecystokinin receptor (HUMCCKR), human-derived
25 neuropeptid receptor (HSNEURA) and the like [Figure 14].

The nucleotide sequence represented by SEQ ID NO: 18
(Figure 15: S3A) is a nucleotide sequence highly homologous
to the DNA sequence coding for the amino acid sequence
corresponding to or near the third membrane-spanning domain
of known G protein coupled receptors such as human-derived
galanin receptor (HUMGALAREC), human-derived CCK-B receptor
(S70057), human-derived ET_A receptor (S67127), human-derived
ET_B receptor (S44866), human-derived C5A receptor (HUMC5AAR),
human-derived angiotensin II receptor (HUMANTIR),
human-derived bradykinin receptor (HUMBK2R), human-derived
neurotensin receptor (HSNEURA), human-derived GRP receptor
(HUMGRPR), human-derived somatostatin 5 receptor (HUMFSRS),

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human-derived IL-8 receptor (HUMIL8RA), human-derived neurokinin 2 (neurokinin A) receptor (HUMNEKAR) and the like [Figure 15].

The nucleotide sequence represented by SEQ ID NO: 19
5 (Figure 16: S6A) is a nucleotide sequence which is complementary to the nucleotide sequence (Figure 16) highly homologous to the DNA sequence coding for the amino acid sequence corresponding to or near the sixth membrane-spanning domain of known G protein coupled receptors such as human-
10 derived galanin receptor (HUMGLAREC), human-derived CCK-B receptor (S70057), human-derived ET_A receptor (S67127), human-derived ET_B receptor (S44866), human-derived C5A receptor (HUMC5AAR), human-derived angiotensin II receptor (HUMANTIR), human-derived bradykinin receptor (HUMBK2R), human-derived
15 neurotensin receptor (HSNEURA), human-derived GRP receptor (HUMGRPR), human-derived somatostatin 5 receptor (HUMFSRS), human-derived IL-8 receptor (HUMIL8RA), human-derived neurokinin 2 (neurokinin A) receptor (HUMNEKAR) and the like [Figure 16].

20 The above-mentioned abbreviations in the parentheses are the identifiers (or reference numbers) which are shown when GenBank/EMBL Data Bank is searched using a DNASIS Gene/Protein Sequence Data Base (CD019; Hitachi Software Engineering, Japan) and are usually called "Accession Numbers" or "Entry Names".
25 HTRHR is, however, the sequence as described in Japanese Patent Application No. Hei 5-286986 (or No. 286986/1993) (EPA 638645).

The DNA (or nucleotides) of the present invention may be manufactured by DNA synthetic methods which are known
30 per se or by methods similar thereto. The DNA (or nucleotides) of the present invention may be an oligonucleotide sequence having 8 to 60 base residues, preferably 12 to 50 base residues, more preferably 15 to 40 residues and most preferably 18 to 30 residues.

35 Among the DNAs of the present invention, the DNA having the nucleotide sequence represented by SEQ ID NO: 1 or

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SEQ ID NO: 12 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA encoding the amino acid sequence corresponding to or near the first membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded (i.e. is hybridizable) with RNA or DNA (including genome DNA, cDNA) coding for the amino acid sequence corresponding to or near the first membrane-spanning domain of known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded (i.e. is hybridizable) with nucleotide sequences encoding other membrane-spanning domains as well.

The DNA having a nucleotide sequence represented by SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 14 or SEQ ID NO: 18 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA coding for the amino acid sequence corresponding to or near the third membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded with RNA or DNA (including genome DNA, cDNA) coding for the part corresponding to or near the third membrane-spanning domain of known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded with nucleotide sequences encoding other membrane-spanning domains as well.

The DNA having a nucleotide sequence represented by SEQ ID NO: 10 or SEQ ID NO: 16 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA coding for the amino acid sequence corresponding to or near the second membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded with RNA or DNA (including genome DNA, cDNA) coding for the part corresponding to or near the second membrane-spanning domain of known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded with nucleotide sequences encoding other membrane-spanning domains as well.

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The DNA having a nucleotide sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17 or SEQ ID NO: 19 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA coding for the amino acid sequence corresponding to or near the sixth membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded with RNA or DNA (including genome DNA, cDNA) coding for the part corresponding to or near the sixth membrane-spanning domain of known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded with nucleotide sequences encoding other membrane-spanning domains as well.

The DNA having a nucleotide sequence represented by SEQ ID NO: 11 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA coding for the amino acid sequence corresponding to or near the seventh membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded with RNA or DNA (including genome DNA, cDNA) coding for the part corresponding to or near the seventh membrane-spanning domain of known or unknown G protein coupled receptor proteins and, further more, it can be complementarily bonded with nucleotide sequences encoding other transmembrane domains as well.

The DNA having a nucleotide sequence represented by SEQ ID NO: 13 is a nucleotide sequence which is commonly present in the nucleotide sequence of the DNA coding for the amino acid sequence corresponding to or near the third membrane-spanning domain of the above-mentioned known G protein coupled receptor protein. Therefore, it can be complementarily bonded with RNA or DNA (including genome DNA, cDNA) coding for the part corresponding to or near the third membrane-spanning domain of known or unknown G protein coupled receptor proteins and, furthermore, it can be complementarily bonded with nucleotide sequences encoding other membrane-spanning domains as well.

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Accordingly, the DNAs (or nucleotides) of the present invention can be used as DNA primers for a polymerase chain reaction (hereinafter, sometimes referred to as PCR).

For example:

5 (i) a polymerase chain reaction is carried out by mixing
 (1) a small amount of DNA (or DNA fragment(s)) which codes for G protein coupled receptor protein, said DNA (or DNA fragment(s)) acting as a template,
10 (2) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1, DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 10, DNA primers having a nucleotide sequence represented by SEQ ID NO: 12, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14, DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and
15 (3) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 11, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19; or
20 (ii) a polymerase chain reaction is carried out by mixing

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(1) a small amount of DNA (or DNA fragment(s)) coding for G protein coupled receptor protein, said DNA (or DNA fragment(s)) acting as a template,
5 (2) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and
(3) at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence
10 represented by SEQ ID NO: 13
so that it is possible to amplify the target DNA (or DNA fragment(s)) coding for said receptor protein.

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 11, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19, said DNA primer(s) is(are) bonded (hybridized) with the nucleotide sequence at the 3'-side of the + chain (plus chain) of template RNA or DNA (or fragment(s) thereof) coding for the sixth membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the - chain (minus chain) proceeds
30 in the 5' → 3' direction.

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the + chain (plus chain) of template RNA or DNA (or fragment(s) thereof) coding for the seventh membrane-spanning domain or other membrane-spanning domains of the G protein

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coupled receptor protein whereupon an elongation of the - chain (minus chain) proceeds in the 5' — 3' direction.

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the - chain (minus chain) of template RNA or DNA (or fragment(s) thereof) coding for the first membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the + chain (plus chain) proceeds in the 5' — 3' direction.

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the - chain (minus chain) of template RNA or DNA (or fragment(s) thereof) coding for the second membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the + chain (plus chain) proceeds in the 5' — 3' direction.

When the PCR is carried out using at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18, said DNA primer is bonded with the nucleotide sequence at the 3'-side of the - chain (minus chain) of template RNA or DNA (or fragment(s) thereof) coding for the third membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor protein whereupon an elongation of the + chain (plus chain) proceeds in the 5' — 3' direction.

Accordingly, when the DNA primers having nucleotide sequences represented by any of SEQ ID NO: 1 to SEQ ID NO: 19 of the present invention are used in combination each other, DNA (or DNA fragment(s)) coding for G protein coupled receptor protein can be successfully amplified.

One embodiment of the present invention provides:

(A) a method of amplifying DNA coding for the G protein coupled receptor protein (e.g., from the first to sixth membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19;

(B) a method of amplifying DNA coding for the G protein coupled receptor protein (e.g., from the first to seventh membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a

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nucleotide sequence represented by SEQ ID NO: 12 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO:11;

5 (C) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the second to sixth membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

10 ① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a 15 nucleotide sequence represented by SEQ ID NO: 16 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a 20 nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a 25 nucleotide sequence represented by SEQ ID NO: 19;

(D) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the second to seventh membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in 30 that a polymerase chain reaction is carried out by mixing

① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence 35 represented by SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and

③ at least one DNA primer selected from the group

consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11;

(E) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the third to sixth

5 membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

10 ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

15 ③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19;

20 (F) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the third to seventh membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

25 ① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

30 ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence

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represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7,

5 DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11; and

10 (G) a method of amplifying a DNA coding for the G protein coupled receptor protein (e.g., from the first to third membrane-spanning (transmembrane) domains or other segments of the G protein coupled receptor protein), characterized in that a polymerase chain reaction is carried out by mixing

15 ① a DNA coding for the G protein coupled receptor protein, said DNA acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

20 ③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 13.

25 An example of more preferred combination of the DNA primers in the amplification according to the above-mentioned (A) includes a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2 and the like.

30 An example of more preferred combination of the DNA primers in the amplification according to the above-mentioned (D) includes a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 10 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 11 and the like.

35 An example of more preferred combination of the DNA

primers in the amplification according to the above-mentioned (E) includes:

(i) a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 5 or a DNA primer having a nucleotide sequence represented by SEQ ID NO: 6 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 8 or a DNA primer having a nucleotide sequence represented by SEQ ID NO: 9;

5 (ii) a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 3 or a DNA primer having a nucleotide sequence represented by SEQ ID NO: 7 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 4 and the like.

10

An example of more preferred combination of the DNA primers in the amplification according to the above-mentioned (G) includes a combination of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 12 with a DNA primer having a nucleotide sequence represented by SEQ ID NO: 13 and the like.

The amplification may be carried out in accordance 20 with known PCR techniques. For example, it may be carried out by the method described in Saiki, R. K. et al., Science, 239:487-491 (1988). Temperature, time, buffer, number of reaction cycles, enzyme such as DNA polymerase, addition of 2'-deoxy-7-deazaguanosine triphosphate or inosine, etc.

25

in the PCR amplification may be suitably selected depending upon the type of target DNA and other factors.

When RNA is used as a template, PCR amplification may be carried out, for example, by the method described in Saiki, R. K. et al., Science, 239:487-491(1988).

30 Moreover, the DNA having a nucleotide sequence represented by SEQ ID NO: 1 or SEQ ID NO: 12 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the 3'-side of the - chain of the DNA coding for the amino acid sequence 35 corresponding to or near the first membrane-spanning domain of the G protein coupled receptor protein; the DNA having a nucleotide sequence represented by SEQ ID NO: 10 or SEQ ID

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NO: 16 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the 3'-side of the - chain of the DNA coding for the amino acid sequence corresponding to or near the second membrane-spanning domain of the G protein coupled receptor protein; the DNA having a nucleotide sequence represented by SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 14 or SEQ ID NO: 18 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the 3'-side of the - chain of the DNA coding for the amino acid sequence corresponding to or near the third membrane-spanning domain of the G protein coupled receptor protein; the DNA having a nucleotide sequence represented by SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17 or SEQ ID NO: 19 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the 3'-side of the + chain of the DNA coding for the amino acid sequence corresponding to or near the sixth membrane-spanning domain of the G protein coupled receptor protein; the DNA having a nucleotide sequence represented by SEQ ID NO: 11 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the 3'-side of the + chain of the DNA coding for the amino acid sequence corresponding to or near the third membrane-spanning domain of the G protein coupled receptor protein; and the DNA having a nucleotide sequence represented by SEQ ID NO: 13 of the present invention can be selectively and complementarily bonded (hybridized) with the nucleotide sequence at the 3'-side of the + chain of the DNA coding for the amino acid sequence corresponding to or near the third membrane-spanning domain of the G protein coupled receptor protein and, accordingly, said DNA is also advantageously useful as a probe for screening DNA libraries for DNA (or DNA fragment(s)) encoding part or all of the polypeptide sequence of G protein coupled receptor proteins.

These screening methods for DNA (or DNA fragment(s))

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encoding part or all of the polypeptide sequence of G protein coupled receptor proteins from the DNA library by using as a reagent, because it can be used as a probe the DNA of the present invention may be carried out according to DNA cloning 5 methods known per se by those of skill in the art or methods similar thereto. Especially when the DNA of the present invention is used as a DNA primer for the PCR, both amplification and screening of the DNA (or DNA fragment) coding for the G protein coupled receptor protein can be conducted in 10 a single step.

Thus, when the DNAs of the present invention are suitably combined and used as the DNA primer for the PCR, said DNA primer(s) is(are) bonded (hybridized) with RNA or DNA (or fragment(s) thereof) encoding the amino acid sequence 15 of the first membrane-spanning (transmembrane) domain, the second membrane-spanning domain, the third membrane-spanning domain, the sixth membrane-spanning domain, the seventh membrane-spanning domain or other membrane-spanning domains of G protein coupled receptor proteins to amplify, for example, 20 ① RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning to the sixth membrane-spanning domains of G protein coupled receptor proteins,

② RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning to the 25 seventh membrane-spanning domains of G protein coupled receptor proteins,

③ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the third membrane-spanning to the sixth membrane-spanning domains of G protein coupled receptor 30 proteins,

④ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the third membrane-spanning to the seventh membrane-spanning domains of G protein coupled receptor 35 proteins,

⑤ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the second membrane-spanning to the

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sixth membrane-spanning domains of G protein coupled receptor proteins or RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of other domains thereof,

5 ⑥ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the second membrane-spanning to the seventh membrane-spanning domains of G protein coupled receptor proteins,

10 ⑦ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning to the third membrane-spanning domains of G protein coupled receptor proteins or

15 ⑧ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence of other domains of G protein coupled receptor proteins.

Through using the DNA primer according to the present invention, therefore, selective amplifications of:

20 ① RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the first membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor proteins;

25 ② RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the first membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor proteins;

30 ③ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the third membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor proteins;

35 ④ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the third membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor proteins;

⑤ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the second membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor proteins or RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering other areas thereof,

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⑥ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the second membrane-spanning domain to the seventh membrane-spanning domain of G protein coupled receptor proteins;

5 ⑦ RNA or DNA (or fragment(s) thereof) coding for the amino acid sequence covering from the first membrane-spanning domain to the third membrane-spanning domain of G protein coupled receptor proteins; and the like,
from DNA libraries can be successfully achieved.

10 Among the DNA primers of the present invention,
the combination of

① a DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 or SEQ ID NO: 2; with
② at least one DNA primer selected from the group consisting
15 of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 4, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 8, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 9, a DNA primer
20 having a nucleotide sequence represented by SEQ ID NO: 15,
a DNA primer having a nucleotide sequence represented by SEQ ID NO: 17 and a DNA primer having a nucleotide sequence represented by SEQ ID NO: 19;
is, unlike conventional primers, capable of selectively
25 amplifying a broad area covering from the first membrane-
spanning domain to the sixth membrane-spanning domain or other
domains of G protein coupled receptor proteins.

Among the DNA primers of the present invention, the
combination of

30 ① a DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 or SEQ ID NO: 12; with
② a DNA primer having a nucleotide sequence represented by SEQ ID NO: 11;
is, unlike conventional primers, capable of selectively
35 amplifying a broad area covering from the first membrane-
spanning domain to the seventh membrane-spanning domain or
other domains of G protein coupled receptor proteins.

Among the DNA primers of the present invention, the combination of

- ① a DNA primer having a nucleotide sequence represented by SEQ ID NO: 10 or SEQ ID NO: 16; with
- 5 ② at least one DNA primer selected from the group consisting of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 4, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 8, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 9, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 15, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 17 and a DNA primer having a nucleotide sequence represented by SEQ ID NO: 19;
- 10 15 is, unlike conventional primers, capable of selectively amplifying a broad area covering from the second membrane-spanning domain to the sixth membrane-spanning domain or other domains of G protein coupled receptor proteins.

Among the DNA primers of the present invention, the combination of

- ① a DNA primer having a nucleotide sequence represented by SEQ ID NO:10 or SEQ ID NO:16; with
- 25 ② a DNA primer having a nucleotide sequence represented by SEQ ID NO:11;
- is, unlike conventional primers, capable of selectively amplifying a broad area covering from the second membrane-spanning domain to the seventh membrane-spanning domain or other domains of G protein coupled receptor proteins.

Among the DNA primers of the present invention, the combination of

- ① at least one DNA primer selected from the group consisting of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 3, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 5, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 6, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 7, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 14 and a DNA

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primer having a nucleotide sequence represented by SEQ ID NO: 18; with

② a DNA primer having a nucleotide sequence represented by SEQ ID NO: 11;

5 is, unlike conventional primers, capable of selectively amplifying a broad area covering from the third membrane-spanning domain to the seventh membrane-spanning domain or other domains of G protein coupled receptor proteins.

Therefore, the protein hydrophobicity plotting of G 10 protein coupled receptor proteins and the homology at the amino acid level or the nucleic acid level between G protein coupled receptor proteins and other similar receptor proteins [said hydrophobicity plotting and homology both serve as standards for determining whether or not RNA or DNA (or fragment(s) 15 thereof) obtained according to the present invention is(are) encoding part or all of the amino acid sequence of G protein coupled receptor protein] can now be more clearly calculated.

Among the DNA primers of the present invention, the combination of

20 ① at least one DNA primer selected from the group consisting of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 3, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 5, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 6, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 7, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 14 and a DNA 25 primer having a nucleotide sequence represented by SEQ ID NO: 18; with

② at least one DNA primer selected from the group consisting 30 of a DNA primer having a nucleotide sequence represented by SEQ ID NO: 2, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 4, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 8, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 9, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 15, a DNA primer having a nucleotide sequence represented by SEQ ID NO: 17 and 35 a DNA primer having a nucleotide sequence represented by SEQ ID

NO: 19;

is capable of amplifying the areas covering from the third membrane-spanning domain to the sixth membrane-spanning domain thereof at once like the conventional DNA primers and,
5 moreover, it is capable of more selectively and efficiently amplifying DNA coding for G protein coupled receptor proteins though it has not been obtained through the conventional DNA primers.

Moreover, among the DNA primers of the present
10 invention, the combination of
① at least one DNA primer selected from DNA primers having a nucleotide sequence of SEQ ID NO: 1 and DNA primers having a nucleotide sequence of SEQ ID NO: 12; with
② a DNA primer having a nucleotide sequence represented by
15 SEQ ID NO: 13;
is capable of amplifying the areas covering from the first membrane-spanning domain to the third membrane-spanning domain thereof at once.

Then (a) the amplified DNA (or fragment(s) thereof)
20 coding for the amino acid sequence of from the first membrane-
spanning domain to the sixth membrane-spanning domain of G
protein coupled receptor protein, (b) the amplified DNA (or
fragment(s) thereof) coding for the amino acid sequence of
from the first membrane-spanning domain to the seventh
25 membrane-spanning domain of G protein coupled receptor protein,
(c) the amplified DNA (or fragment(s) thereof) coding for the
amino acid sequence of from the third membrane-spanning domain
to the sixth membrane-spanning domain of G protein coupled
receptor protein, (d) the amplified DNA (or fragment(s)
30 thereof) coding for the amino acid sequence of from the third
membrane-spanning domain to the seventh membrane-spanning
domain of G protein coupled receptor protein, (e) the amplified
DNA (or fragment(s) thereof) coding for the amino acid sequence
of from the second membrane-spanning domain to the sixth
35 membrane-spanning domain of G protein coupled receptor protein,
(f) the amplified DNA (or fragment(s) thereof) coding for the
amino acid sequence of from the second membrane-spanning domain

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to the seventh membrane-spanning domain of G protein coupled receptor protein, (g) the amplified DNA (or fragment(s) thereof) coding for the amino acid sequence of from the first membrane-spanning domain to the third membrane-spanning domain of G protein coupled receptor protein or (h) the amplified DNA (or fragment(s) thereof) coding for the amino acid sequence of other domains of G protein coupled receptor protein may be used as a probe(s) to screen for full-length DNA which completely encodes G protein coupled receptor proteins from 5 DNA libraries according to methods known per se by those of skill in the art or methods similar thereto.

The DNA libraries used in the present invention include any of genome DNA libraries, cDNA libraries and RNA libraries. The term "DNA library" or "DNA libraries" as used 15 herein refers to a DNA library or DNA libraries including all of those libraries.

The present invention further provides screening methods for target DNA (or fragment(s) thereof) coding for G protein coupled receptor protein from the DNA library containing DNA (or fragment(s) thereof) coding for receptor 20 proteins, which comprise employing the DNA of the present invention as a DNA primer for the PCR.

One preferred embodiment of the present invention is a method for cloning full-length DNA which completely encodes 25 an amino acid sequence of G protein coupled receptor protein from DNA libraries which comprises the steps of (i) using the DNA of the present invention as a DNA primer for PCR; (ii) carrying out PCR in the presence of said 30 DNA primer with the DNA library to amplify and select (i.e. screen for) a DNA fragment coding for the amino acid sequence of from the first membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the first membrane-spanning domain to the seventh membrane-spanning 35 domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the third membrane-

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spanning domain to the sixth membrane-spanning domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the third membrane-spanning domain to the seventh membrane-spanning domain of G protein
5 coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the second membrane-spanning domain to the sixth membrane-spanning domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the second membrane-spanning domain to the
10 seventh membrane-spanning domain of G protein coupled receptor protein, a DNA fragment coding for the amino acid sequence of from the first membrane-spanning domain to the third membrane-spanning domain of G protein coupled receptor protein or a DNA fragment coding for other domains of G protein coupled receptor protein; and
15 (iii) cloning said full-length DNA from the DNA library according to cloning methods known per se by those of skill in the art or methods similar thereto by using, as a probe, the DNA fragment obtained in the above step (ii).
20 Preferably, an embodiment of the present invention is a screening method of DNA coding for G protein coupled receptor proteins from DNA libraries, which comprises carrying out a polymerase chain reaction in the presence of a mixture of
25 ① the DNA library,
② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1, DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 10, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14, DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and
30
35

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③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 11, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19 to selectively amplify template DNA coding for G protein coupled receptor protein contained in the DNA library.

More preferably, embodiments of the present invention include:

- (1) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of
 - ① the DNA library,
 - ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and
 - ③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19

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to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;

5 (2) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

10 ① the DNA library,

15 ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

③ at least one DNA primer selected from the group consisting

20 of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11

to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;

25 (3) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

30 ① the DNA library,

35 ② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by

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SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and

③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by

5 SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15,

10 DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19

to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like

15 (e.g. the regions spanning from the second transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;

(4) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

25 ① the DNA library,
② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 10 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 16 and

30 ③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11

35 to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the second transmembrane domain to the seventh transmembrane domain of G protein coupled

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receptor protein or other domains thereof) contained in the DNA library;

(5) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the third transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

10 ① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 18 and

15 ③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 2, DNA primers having a nucleotide sequence represented by SEQ ID NO: 4, DNA primers having a nucleotide sequence represented by SEQ ID NO: 8, DNA primers having a nucleotide sequence represented by SEQ ID NO: 9, DNA primers having a nucleotide sequence represented by SEQ ID NO: 15, DNA primers having a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 19

20 30 to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the third transmembrane domain to the sixth transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library;

35 (6) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like

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(e.g. the regions spanning from the third transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

5 ① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 3, DNA primers having a nucleotide sequence represented by SEQ ID NO: 5, DNA primers having a nucleotide sequence represented by SEQ ID NO: 6, DNA primers having a nucleotide sequence represented by SEQ ID NO: 7, DNA primers having a nucleotide sequence represented by SEQ ID NO: 14 and DNA primers having a nucleotide sequence represented by

10 SEQ ID NO: 18 and

15 ③ at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 11

to selectively amplify the DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the third transmembrane domain to the seventh transmembrane domain of G protein coupled receptor protein or other domains thereof) contained in the DNA library; and

20 (7) a screening method of DNA coding for the amino acid sequence of G protein coupled receptor protein and the like (e.g. the regions spanning from the first transmembrane domain to the third transmembrane domain of G protein coupled receptor protein or other domains thereof) from a DNA library, which comprises carrying out a polymerase chain reaction in the presence of a mixture of

25 ① the DNA library,

② at least one DNA primer selected from the group consisting of DNA primers having a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers having a nucleotide sequence represented by SEQ ID NO: 12 and

30 ③ at least one DNA primer selected from the group consisting

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of DNA primers having a nucleotide sequence represented by
SEQ ID NO: 13
to selectively amplify the DNA coding for the amino acid
sequence of G protein coupled receptor protein and the like
5 (e.g. the regions spanning from the first transmembrane
domain to the third transmembrane domain of G protein coupled
receptor protein or other domains thereof) contained in
the DNA library.

Particularly preferably, embodiments of the present
10 invention include:

(8) a screening method of DNA coding for the amino acid
sequence of G protein coupled receptor protein from a DNA
library, which comprises carrying out a polymerase chain
reaction in the presence of a mixture of
15 ① the DNA library,
② a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 1 and
③ a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 2
20 to selectively amplify the DNA coding for the amino acid
sequence of G protein coupled receptor protein contained in
the DNA library;
(9) a screening method of DNA coding for the amino acid
sequence of G protein coupled receptor protein from a DNA
25 library, which comprises carrying out a polymerase chain
reaction in the presence of a mixture of
① the DNA library,
② a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 3 and
30 ③ a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 4
to selectively amplify the DNA coding for the amino acid
sequence of G protein coupled receptor protein contained in
the DNA library;
35 (10) a screening method of DNA coding for the amino acid
sequence of G protein coupled receptor protein from a DNA
library, which comprises carrying out a polymerase chain

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reaction in the presence of a mixture of

① the DNA library,

② a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 6 and

5 ③ a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 8

to selectively amplify the DNA coding for the amino acid
sequence of G protein coupled receptor protein contained in
the DNA library; and

10 (11) a screening method of DNA coding for the amino acid
sequence of G protein coupled receptor protein from a DNA
library, which comprises carrying out a polymerase chain
reaction in the presence of a mixture of

① the DNA library,

15 ② a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 10 and

③ a DNA primer having a nucleotide sequence represented by
SEQ ID NO: 11

20 to selectively amplify the DNA coding for the amino acid
sequence of G protein coupled receptor protein contained in
the DNA library.

The cloned DNAs can be analyzed, usually by
restriction enzyme analysis and/or sequencing.

Target RNA or DNA (or fragment(s) thereof) coding
25 for G protein coupled receptor protein in the amplification
and the screening by the PCR techniques wherein the DNA of
the present invention is employed may include RNA, DNA or
fragments thereof coding for known (or prior art) G protein
coupled receptor proteins and RNA, DNA or fragments thereof
30 coding for unknown (novel) G protein coupled receptor
proteins.

These target RNA or DNA (or fragment(s) thereof) may
include novel nucleotide sequences and even known nucleotide
sequences.

35 Examples of such nucleotide sequences are RNA or DNA
(or fragment(s)) coding for a G protein coupled receptor
protein, said RNA or DNA (or fragment(s)) being derived from

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all cells and tissues (e.g. pituitary gland, brain, pancreas, lung, adrenal gland, etc.) of vertebrate animals (e.g. mice, rats, cats, dogs, swines, cattle, horses, monkeys, human beings, etc.), insects or other invertebrate animals (e.g. 5 drosophilae, silkworms, Barathra brassicae, etc.), plants (e.g. rice plant, wheat, tomato, etc.) and cultured cell lines derived therefrom, etc.

Specific examples of the nucleotide sequences are RNA or DNA (or fragment(s)) coding for G protein coupled 10 receptor proteins such as receptor proteins to angiotensin, bombesin, canavinoïd, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive intestinal and related peptide), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP 15 (calcitonin gene related peptide), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenaline, α - and β -chemokine (IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, 20 histamine, neurotensin, TRH, pancreatic polypeptide, galanin, family members thereof, etc.

In the PCR amplification using the DNA of the present invention, the DNA (or DNA fragment) acting as a template may include any DNA so far as it is derived from the 25 above-mentioned tissues and cells. More specifically, the template DNA (or DNA fragment) includes any of genome DNA, genome DNA libraries, cDNA derived from the tissues and cells and cDNA libraries derived from the tissues and cells. cDNA libraries derived from human tissues and cells are particularly suitable. Vectors to be used in the DNA library 30 may include any of bacteriophages, plasmids, cosmids, phagimids, etc. It is also possible to directly amplify the template DNA (or DNA fragment) by reverse transcriptase polymerase chain reaction (RT-PCR) techniques using mRNA 35 fractions prepared from the tissues and cells. The DNA which is to be a template may be either DNA completely coding for G protein coupled receptor proteins or DNA fragments (or

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segments) thereof.

Preferably, the RNA or DNA (or fragment(s) thereof) obtained via the instant screening method for G protein coupled receptor protein coding DNA wherein said method uses the DNA according to the present invention is a G protein coupled receptor protein-encoding RNA or DNA (or fragment(s) thereof) contained in the used DNA library. More specifically, it is an RNA or DNA (or RNA fragment(s) or DNA fragment(s) (hereinafter, may be often abbreviated as just "DNA") coding for G protein coupled receptor proteins such as angiotensin receptor, bombesin receptor, canavinoind receptor, cholecystokinin receptor, glutamine receptor, serotonin receptor, melatonin receptor, neuropeptide Y receptor, opioid receptor, purine receptor, vasopressin receptor, oxytocin receptor, VIP receptor (vasoactive intestinal and related peptide receptor), somatostatin receptor, dopamine receptor, motilin receptor, amylin receptor, bradykinin receptor, CGRP receptor (calcitonin gene related peptide receptor), adrenomedullin receptor, leukotriene receptor, pancreastatin receptor, prostaglandin receptor, thromboxane receptor, adenosine receptor, adrenaline receptor, α - and β -chemokine receptor (receptors to IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin receptor, enterogastrin receptor, histamine receptor, neuropeptidin receptor, TRH receptor, pancreatic polypeptide receptor, galanin receptor, their family member receptors, etc.

When the DNA obtained by the screening method of the present invention is the DNA fragment which partially codes for a G protein coupled receptor protein, it is possible to isolate DNA completely encoding said G protein coupled receptor protein from a suitable DNA library according to cloning techniques known per se by using said DNA fragment as a probe.

Means for cloning the DNA completely encoding G protein coupled receptor proteins may include a PCR amplification employing a synthetic DNA primer having the partial nucleotide sequence of the DNA fragment partially

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coding for the G protein coupled receptor protein and a selection of the target DNA via a hybridization with DNA or synthetic DNA having part or all of the region of said DNA fragments. The hybridization may be conducted, for example, 5 by the methods described in Molecular Cloning, 2nd ed.; J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989. When the commercially available library is used, it may be conducted according to the manners described in the protocols attached thereto.

10 The DNA completely encoding G protein coupled receptor protein (full-length G protein coupled receptor protein DNA) may be used, depending upon its object, either as it is or after digesting with a restriction enzyme or after ligating with a linker if desired. Said DNA may have 15 ATG at the 5'-terminal as the translation initiation codon and TAA, TGA or TAG at the 3' terminal as the translation termination codon. These translation initiation codons and translation termination codons may be added using a suitable synthetic DNA adaptor. In addition, it is possible to 20 determine said receptor protein-expressing tissues/cells by northern blottings using said DNA as a probe. It is also possible to express target receptor proteins by introducing DNA having the entire coding region of the receptor protein into animal cells after binding with a suitable promoter.

25 The G protein coupled receptor protein according to the present invention is a G protein coupled receptor protein encoded by the G protein coupled receptor protein-encoding DNA obtained by the screening method of the present invention. More specifically, the G protein coupled receptor 30 protein according to the present invention includes G protein coupled receptor proteins such as angiotensin receptor protein, bombesin receptor protein, canavinoind receptor protein, cholecystokinin receptor protein, glutamine receptor protein, serotonin receptor protein, melatonin receptor protein, 35 neuropeptide Y receptor protein, opioid receptor protein, purine receptor protein, vasopressin receptor protein, oxytocin receptor protein, VIP receptor protein (vasoactive

intestinal and related peptide receptor protein), somatostatin receptor protein, dopamine receptor protein, motilin receptor protein, amylin receptor protein, bradykinin receptor protein, CGRP receptor protein (calcitonin gene related peptide receptor protein), adrenomedullin receptor protein, leukotriene receptor protein, pancreastatin receptor protein, prostaglandin receptor protein, thromboxane receptor protein, adenosine receptor protein, adrenaline receptor protein, α - and β -chemokine receptor protein (receptor protein responsive to IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin receptor protein, enterogastrin receptor protein, histamine receptor protein, neuropeptid Y receptor protein, TRH receptor protein, pancreatic polypeptide receptor protein, galanin receptor protein, family members thereof, etc.

According to the present invention, novel G protein coupled receptors proteins, peptide segments or fragments derived from the G protein coupled receptor protein, modified derivatives or analogues thereof, and salts thereof may be recognized, cloned, produced, isolated or characterized.

These G protein coupled receptor proteins are those derived from all cells and tissues (e.g. pituitary gland, pancreas, brain, kidney, liver, gonad, thyroid gland, cholecyst, bone marrow, adrenal, skin, muscle, lung, digestive duct, blood vessel, heart, etc.) of warm-blooded animals (e.g. guinea pig, rat, mouse, swine, sheep, cattle, monkey, human beings, rabbit, cat, dog, horse, etc.), and any of proteins as long as they comprise an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27, an amino acid sequence represented by SEQ ID NO: 28, an amino acid sequence represented by SEQ ID NO: 34, an amino acid sequence represented by SEQ ID NO: 35, an amino acid sequence represented by SEQ ID NO: 38, an amino acid sequence represented by SEQ ID NO: 39, an amino acid sequence

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represented by SEQ ID NO: 56, and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 38, SEQ ID NO: 39, and/or SEQ ID 5 NO: 56.

In one embodiment of the present invention, G protein coupled receptor proteins are those derived from all cells and tissues (e.g. pituitary gland, pancreas, brain, kidney, liver, gonad, thyroid gland, cholecyst, bone marrow, adrenal, skin, 10 muscle, lung, digestive duct, blood vessel, heart, etc.) of warm-blooded animals (e.g. guinea pig, rat, mouse, swine, sheep, cattle, monkey, human beings, cat, dog, horse, etc.), and any of proteins as long as they comprise an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence 15 represented by SEQ ID NO: 25, an amino acid sequence represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27, an amino acid sequence represented by SEQ ID NO: 28, and substantial equivalents to 20 the amino acid sequence represented by SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, or SEQ ID NO: 28. These G protein coupled receptor proteins may include proteins having an amino acid sequence selected from the group 25 consisting of an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27 and an amino acid sequence represented by SEQ ID NO: 28, proteins wherein the amino acid sequence thereof is about 90% to 99.9% homologous 30 to an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27 or an amino acid sequence represented by SEQ ID NO: 28 and the activity thereof is 35 substantially equivalent to the protein having an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence

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represented by SEQ ID NO: 26, an amino acid sequence
represented by SEQ ID NO: 27 or an amino acid sequence
represented by SEQ ID NO: 28 and the like. The substantially
equivalent activity may include ligand binding activity,
5 signal information transmitting, etc. The term "substantially
equivalent" or "substantial equivalent" means that the nature
of the ligand binding activity and the like is equivalent.
Therefore, it is allowable that even differences among grades
such as ligand binding affinity grades and ligand binding
10 activity grades and quantitative factors such as molecular
weights of receptor proteins are present.

In another embodiment of the present invention, G
protein coupled receptor proteins include human pituitary
gland-derived G protein coupled receptor proteins comprising
15 an amino acid sequence selected from the group consisting of
an amino acid sequence represented by SEQ ID NO: 24, and/or
an amino acid sequence represented by SEQ ID NO: 25, mouse
pancreas-derived G protein coupled receptor proteins comprising
an amino acid sequence represented by SEQ ID NO: 27, mouse
20 pancreas-derived G protein coupled receptor proteins comprising
an amino acid sequence represented by SEQ ID NO: 28, etc.
Examples of the human pituitary gland-derived G protein coupled
receptor protein comprising an amino acid sequence selected
from the group consisting of an amino acid sequence represented
25 by SEQ ID NO: 24, and an amino acid sequence represented by SEQ
ID NO: 25, are human pituitary gland-derived G protein coupled
receptor proteins comprising an amino acid sequence represented
by SEQ ID NO: 24, etc. These G protein coupled receptor
proteins may include proteins wherein one or more amino acid
30 residues (preferably from 2 to 30 amino acid residues, more
preferably from 2 to 10 amino acid residues) are deleted from
the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 25, SEQ
ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28, proteins wherein one
35 or more amino acid residues (preferably from 2 to 30 amino acid
residues, more preferably from 2 to 10 amino acid residues) are
added to the amino acid sequence of SEQ ID NO: 24, SEQ ID NO:
25, SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28, proteins

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wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27 or SEQ ID NO: 28, are 5 substituted with one or more amino acid residues, etc.

In yet another embodiment of the present invention, G protein coupled receptor proteins include those derived from all cells and tissues (e.g. amygdaloid nucleus, pituitary gland, pancreas, brain, kidney, liver, gonad, thyroid gland, 10 cholecyst, bone marrow, lung, digestive duct, blood vessel, heart, thymus, spleen, leukocyte, etc.) of warm-blooded animals (e.g. guinea pig, rat, mouse, pig, sheep, cattle, monkey, human beings, etc.), and any of proteins as long as they comprise an amino acid sequence selected from the group 15 consisting of an amino acid sequence represented by SEQ ID NO: 34 and/or an amino acid sequence represented by SEQ ID NO: 35. These G protein coupled receptor proteins may include proteins having an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 34 or/and an amino acid sequence represented by SEQ ID NO: 35, proteins wherein the amino acid sequence thereof is 20 about 90% to 99.9% homologous to an amino acid sequence represented by SEQ ID NO: 34 or/and an amino acid sequence represented by SEQ ID NO: 35 and the activity thereof is substantially equivalent to the protein having an amino acid 25 sequence represented by SEQ ID NO: 34 and/or an amino acid sequence represented by SEQ ID NO: 35, and the like. The substantially equivalent activity may include ligand binding activity, signal information transmitting, etc. The term 30 "substantially equivalent" or "substantial equivalent" means that the nature of the ligand binding activity and the like is equivalent. Therefore, it is allowable that even differences among grades such as ligand binding affinity grades and ligand binding activity grades and quantitative factors such as 35 molecular weights of receptor proteins are present.

Examples of the G protein coupled receptor protein are human amygdaloid nucleus-derived G protein coupled receptor proteins

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having an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 34 and/or an amino acid sequence represented by SEQ ID NO: 35, etc. These G protein coupled receptor proteins may include
5 proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 34 or SEQ ID NO: 35, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 34 or SEQ ID NO:
10 35, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of
15 SEQ ID NO: 34 or SEQ ID NO: 35, are substituted with one or more amino acid residues, etc.

In still another embodiment of the present invention, these G protein coupled receptor proteins are those derived from all cells and tissues (e.g. amygdaloid nucleus, pituitary body, pancreas, brain, kidney, liver, gonad, thyroid gland, cholecyst, bone marrow, lung, digestive duct, blood vessel, heart, thymus, leukocyte, etc.) of warm-blooded animals (e.g. guinea pig, rat, mouse, swine, sheep, cattle, monkey, human beings, etc.), and any of proteins as long as they
20 comprise an amino acid sequence represented by SEQ ID NO: 38, or substantial equivalents to the amino acid sequence represented by SEQ ID NO: 38, preferably an amino acid sequence represented by SEQ ID NO: 39, or substantial equivalents to the amino acid sequence represented by SEQ ID NO: 39.
25 These G protein coupled receptor proteins may include proteins having an amino acid sequence represented by SEQ ID NO: 38, proteins wherein the amino acid sequence thereof is about 90% to 99.9% homologous to an amino acid sequence represented by SEQ ID NO: 38 and the activity thereof is substantially equivalent to the protein having an amino acid sequence represented by SEQ ID NO: 38 and the like.
30 These G protein coupled receptor proteins are preferably
35

proteins having an amino acid sequence represented by SEQ ID NO: 39, proteins wherein the amino acid sequence thereof is about 90% to 99.9% homologous to an amino acid sequence represented by SEQ ID NO: 39 and the activity thereof is substantially equivalent to the protein having an amino acid sequence represented by SEQ ID NO: 39, etc. The substantially equivalent activity may include ligand binding activity, signal information transmitting, etc. The term "substantially equivalent" or "substantial equivalent" means that the nature of the ligand binding activity and the like is equivalent. Therefore, it is allowable that even differences among grades such as ligand binding affinity grades and ligand binding activity grades and quantitative factors such as molecular sizes or weights of receptor proteins are present.

It is suggested by data that the mouse pancreatic β -cell strain, MIN6-derived receptor protein of the present invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by pMAH2-17) is a novel purinoceptor subtype which is clearly distinct from prior art purinoceptors.

In another more specific embodiment of the present invention, G protein coupled receptor proteins include mouse pancreatic β -cell line, MIN6, derived G protein coupled receptor proteins comprising an amino acid sequence represented by SEQ ID NO: 38, mouse pancreatic β -cell line, MIN6, derived G protein coupled receptor proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 38, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 38, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are substituted with other amino acid residues in the amino acid sequence of SEQ ID NO: 38, etc. Further preferably these G protein coupled receptor proteins include mouse pancreatic β -cell line, MIN6, derived G

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protein coupled receptor proteins comprising an amino acid sequence represented by SEQ ID NO: 39, mouse pancreatic β -cell line, MIN6, derived G protein coupled receptor proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 39, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 39, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 39 are substituted with other amino acid residues, etc.

In still another embodiment of the present invention, these G protein coupled receptor proteins are those derived from all cells and tissues (e.g. placenta, gonad, amygdaloid nucleus, pituitary body, pancreas, brain, kidney, liver, thyroid gland, cholecyst, bone marrow, lung, digestive duct, blood vessel, heart, thymus, leukocyte, etc.) of human beings, and any of proteins as long as they comprise an amino acid sequence represented by SEQ ID NO: 56, or substantial equivalents to the amino acid sequence represented by SEQ ID NO: 56. These G protein coupled receptor proteins may include proteins having an amino acid sequence represented by SEQ ID NO: 56, proteins wherein the amino acid sequence thereof is about 90% to 99.9% homologous to an amino acid sequence represented by SEQ ID NO: 56 and the activity thereof is substantially equivalent to the protein having an amino acid sequence represented by SEQ ID NO: 56 and the like. The substantially equivalent activity may include ligand binding activity, signal information transmitting, etc. The term "substantially equivalent" or "substantial equivalent" means that the nature of the ligand binding activity and the like is equivalent. Therefore, it is allowable that even differences among grades such as ligand binding affinity grades and ligand binding activity grades and quantitative factors

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such as molecular sizes or weights of receptor proteins are present.

In another more specific embodiment of the present invention, G protein coupled receptor proteins include G 5 protein coupled receptor proteins comprising an amino acid sequence represented by SEQ ID NO: 56, G protein coupled receptor proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are deleted from the amino acid sequence of SEQ ID NO: 56, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) are added to the amino acid sequence of SEQ ID NO: 10 56, proteins wherein one or more amino acid residues (preferably from 2 to 30 amino acid residues, more preferably from 2 to 10 amino acid residues) in the amino acid sequence of SEQ ID NO: 56, are substituted with other 15 amino acid residues, etc.

A portion of the amino acid sequence may be modified 20 (e.g. addition, deletion, substitution with other amino acids, etc.) in the G protein coupled receptor proteins of the present invention.

Furthermore, the G protein coupled receptor proteins 25 of the present invention includes those wherein N-terminal Met is protected with a protecting group (e.g., C₁₋₆ acyl group such as formyl, acetyl, etc.), those wherein the N-terminal side of Glu is cleaved in vivo to make said Glu pyroglutaminated, those wherein the intramolecular side chain 30 of amino acids is protected with a suitable protecting group (e.g., C₁₋₆ acyl group such as formyl, acetyl, etc.), conjugated proteins such as so-called "glycoproteins" wherein saccharide chains are bonded, etc.

The salt of said G protein coupled receptor protein 35 of the present invention includes preferably physiologically acceptable acid addition salts. Examples of such salts are salts thereof with inorganic acids (e.g. hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid, etc.), salts

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thereof with organic acids (e.g. acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid, etc.), etc.

5 The G protein coupled receptor protein or its salt of the present invention may be manufactured from the tissues or cells of warm-blooded animals by purifying methods which are known per se by those skilled in the art or methods similar thereto or may be manufactured by culturing the transformant (or transfectant) (as described herein below) containing G protein coupled receptor protein encoding DNA . The protein or its salt of the present invention may be manufactured by the peptide synthesis as described herein below.

10 The G protein coupled receptor protein fragment (the partial peptide of said G protein coupled receptor protein) may include, for example, the site which is exposed outside cell membranes, among the G protein coupled receptor protein molecule. Examples of the fragment are peptides containing a region which is analyzed as an extracellular area (hydrophilic region or site) in a hydrophobic plotting analysis on the G protein coupled receptor protein represented by any of Figures 24, 25, 28, 31, 32, 36, 38, 41, 44, 47, 50, 53, 57, 58, 59, 64, 70, 74, and 78. A peptide which partly contains a hydrophobic region or site may be used as well. Further, a peptide which separately contains each domain may be used too although the partial peptide (peptide fragment) which contains plural domains at the same time will be used as well.

15 The salt of said G protein coupled receptor protein fragment (partial peptide thereof) includes preferably physiologically acceptable acid addition salts. Examples of such salts are salts thereof with inorganic acids (e.g. hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid, etc.), salts thereof with organic acids (e.g. acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic

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acid, etc.), etc.

The G protein coupled receptor protein fragment (the partial peptide of the G protein coupled receptor protein) may be manufactured by synthesizing methods for peptides which are known per se by those skilled in the art or methods similar thereto or by cleaving (digesting) G protein coupled receptor proteins by a suitable peptidase. Methods of synthesizing peptide may be any of a solid phase synthesis and a liquid phase synthesis. Thus, a partial peptide (peptide fragment) or amino acids which can construct the protein of the present invention is condensed with the residual part thereof and, when the product has a protective group, said protective group is detached whereupon a desired peptide can be manufactured. Examples of the known methods for condensation and for detachment of protective groups include the following

① to ⑤ :

- ① M. Bodanszky and M. A. Ondetti: Peptide Synthesis, Interscience Publishers, New York (1966).
- ② Schroeder and Luebke: The Peptide, Academic Press, New York, 1965.
- ③ Nobuo Izumiya et al.: Fundamentals and Experiments of the Peptide Synthesis, Maruzen KK, Japan (1975).
- ④ Haruaki Yajima and Shumpei Sakakibara: "Seikagaku Jikken Koza 1" (Experiments of Biochemistry, Part 1), "Tanpakusitu No Kagaku IV" (Chemistry of Protein, IV), p.205 (1977), Japan.
- ⑤ Haruaki Yajima (ed): Development of Pharmaceuticals (Second Series), Vol. 14, Peptide Synthesis, Hirokawa Shoten, Japan.

After the reaction, conventional purifying techniques such as salting-out, extraction with solvents, distillation, column chromatography, liquid chromatography, electrophoresis, recrystallization, etc. are optionally combined so that the protein of the present invention can be purified and isolated. When the protein obtained as such is a free compound, it may be

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converted to a suitable salt by known methods while, when it is obtained as a salt, the salt may be converted to a free compound or other salt compounds by known methods.

Furthermore, the product may be manufactured by
5 culturing the transformant (transfected) containing the DNA coding for said partial peptide.

The G protein coupled receptor protein-encoding DNA obtained by the above-mentioned screening method using the DNA of the present invention and the G protein coupled receptor protein encoded by said DNA or the peptide fragment (partial peptide thereof) encoded by said DNA may, for example, be used
10 for the determination of a ligand to said G protein coupled receptor protein or for the screening of a compound which inhibits the binding of said protein coupled receptor protein
15 with a ligand.

In that case, an expression system for the G protein coupled receptor protein-encoding DNA is at first constructed. Hosts for said DNA may be any of animal cells, insect cells, yeasts, Bacillus subtilis, Escherichia coli, etc.

20 Promoters used therefor may be anyone so far as it is suitable as a promoter for the host used for gene expression. Incidentally, the utilization of enhancers for expression is effective as well.

Then the expressing cells per se which are constructed
25 to express the G protein coupled receptor protein or the cell membrane fractions prepared therefrom by methods known per se by those skilled in the art or methods similar thereto may be subjected to a variety of receptor binding experiments.

Ligands used therefor may include any of compounds labeled by
30 a commercially available radioisotope, etc., culture supernatants and tissue extracts which are directly labeled by a chloramine T method or by a lactoperoxidase method.

Separation of bonded or free ligands may be carried out by a direct washing when cells adhered to substrates are used,
35 while, in the case of floating cells or cell membrane fractions thereof, it may be carried out by means of centrifugal separation or filtration. Nonspecific binding with container,

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etc. may be estimated by addition of unlabeled ligands which are about 100 times as much concentrated relatively to the poured labeled ligand.

5 The ligand which is obtained by such a receptor binding experiment may be subjected to a discrimination of agonist versus antagonist.

To be more specific, a natural substance or compound which is presumed to be a ligand with the G protein coupled receptor protein-expressing cell is cultured and, after that, 10 the culture supernatant liquid is collected or the cell is extracted. A change in the components contained therein is measured by, for example, a commercially available measuring kit (e.g. kits for cAMP, diacylglycerol, cGMP, protein kinase A, etc.). Alternatively, it is possible to measure 15 physiological responses such as liberation of Fura-2, [³H]arachidonic acid and [³H]inositol phosphate metabolites by methods known per se by those skilled in the art or methods similar thereto. The compound or natural substance which is obtained by such a screening is an agonist for said G 20 protein coupled receptor protein or an antagonist for said G protein coupled receptor protein and is presumed to act on the tissues and cells in which said receptor is distributed.

Accordingly, it is possible to check the pharmaceutical response (pharmaceutical effect) more efficiently by referring 25 to the distribution disclosed (clarified) by a northern blotting or the like. Moreover, a development of compounds having a novel pharmaceutical response (pharmaceutical effect) in, for example, central nervous tissues, circulatory system, kidney, pancreas, etc. is expected. An efficient development 30 of pharmaceuticals can be proceeded by amplifying G protein coupled receptor protein-encoding DNA selectively from tissues.

The G protein coupled receptor protein-encoding DNA of the present invention may be any coding DNA as long as it contains a nucleotide sequence coding for a G protein 35 coupled receptor protein which contains an amino acid sequence substantially equivalent to the amino acid sequence having SEQ ID NO: 24 and/or which has an activity substantially

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equivalent to the amino acid sequence having SEQ ID NO: 24,
a G protein coupled receptor protein which contains an amino
acid sequence substantially equivalent to the amino acid
sequence having SEQ ID NO: 25 and/or which has an activity
5 substantially equivalent to the amino acid sequence having SEQ
ID NO: 25, a G protein coupled receptor protein which contains
an amino acid sequence substantially equivalent to the amino
acid sequence having SEQ ID NO: 26 and/or which has an activity
substantially equivalent to the amino acid sequence having SEQ
10 ID NO: 26, a G protein coupled receptor protein which contains
an amino acid sequence substantially equivalent to the amino
acid sequence having SEQ ID NO: 27 and/or which has an activity
substantially equivalent to the amino acid sequence having SEQ
ID NO: 27, or a G protein coupled receptor protein which
15 contains an amino acid sequence substantially equivalent to the
amino acid sequence having SEQ ID NO: 28 and/or which has an
activity substantially equivalent to the amino acid sequence
having SEQ ID NO: 28.

Still the G protein coupled receptor protein-encoding
20 DNA of the present invention may be any coding DNA as long as
it contains a nucleotide sequence coding for a G protein
coupled receptor protein which contains an amino acid sequence
substantially equivalent to the amino acid sequence having
SEQ ID NO: 34 and/or which has an activity substantially
25 equivalent to the amino acid sequence having SEQ ID NO: 34,
or a G protein coupled receptor protein which contains an amino
acid sequence substantially equivalent to the amino acid
sequence having SEQ ID NO: 35 and/or which has an activity
substantially equivalent to the amino acid sequence having SEQ
30 ID NO: 35.

Yet the G protein coupled receptor protein-encoding
DNA of the present invention may be any coding DNA as long as
it contains a nucleotide sequence coding for a G protein
coupled receptor protein which contains an amino acid sequence
35 substantially equivalent to the amino acid sequence having
SEQ ID NO: 38 and/or which has an activity substantially
equivalent to the amino acid sequence having SEQ ID NO: 38, or

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preferably a G protein coupled receptor protein which contains an amino acid sequence substantially equivalent to the amino acid sequence having SEQ ID NO: 39 and/or which has an activity substantially equivalent to the amino acid sequence having SEQ
5 ID NO: 39.

Yet the G protein coupled receptor protein-encoding DNA of the present invention may be any coding DNA as long as it contains a nucleotide sequence coding for a G protein coupled receptor protein which contains an amino acid sequence substantially equivalent to the amino acid sequence having SEQ ID NO: 56 and/or which has an activity substantially equivalent to the amino acid sequence having SEQ ID NO: 56, or preferably a G protein coupled receptor protein which contains an amino acid sequence substantially equivalent to the amino acid sequence having SEQ ID NO: 56 and/or which has an activity substantially equivalent to the amino acid sequence having SEQ ID NO: 56.
10
15

The DNA of the present invention may be any one of a human genome DNA, a human genome DNA library, a human tissue and cell-derived cDNA, a human tissue and cell-derived cDNA library and a synthetic DNA. The vector used for the library may include bacteriophage, plasmid, cosmid, phagemid, etc. The DNA can be further amplified directly by the reverse transcriptase polymerase chain reaction (hereinafter briefly referred to as "RT-PCR") using mRNA fractions prepared from tissues and cells.
20
25

In an embodiment, the DNA coding for the human pituitary gland-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 24 includes DNA having a nucleotide sequence represented by SEQ ID NO: 29, etc. The DNA coding for the human pituitary gland-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 25 includes DNA having a nucleotide sequence represented by SEQ ID NO: 30, etc. The DNA coding for the human pituitary gland-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 26 includes DNA having a nucleotide sequence represented by
30
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SEQ ID NO: 31, etc. The DNA coding for the mouse pancreas-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 27 includes DNA having a nucleotide sequence represented by SEQ ID NO: 32, etc. The DNA coding for
5 the mouse pancreas-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 28 includes DNA having a nucleotide sequence represented by SEQ ID NO: 33, etc.

In another embodiment, the DNA coding for the human
10 amygdaloid nucleus-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 34 includes DNA having a nucleotide sequence represented by SEQ ID NO: 36, etc. The DNA coding for the human amygdaloid nucleus-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 35 includes DNA having a nucleotide sequence represented by SEQ ID NO: 37, etc. The DNA coding for the human amygdaloid nucleus-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 34 or the amino acid sequence of SEQ ID NO: 35 includes DNA
15 having a nucleotide sequence represented by SEQ ID NO: 36, DNA having a nucleotide sequence represented by SEQ ID NO: 37, etc. Still in another embodiment, the DNA coding for the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO:
20 38 includes DNA having a nucleotide sequence represented by SEQ ID NO: 40, etc. The DNA coding for the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 39 includes DNA having a nucleotide sequence represented by SEQ ID NO: 41,
25 etc. Yet in another embodiment, the DNA coding for the human-derived G protein coupled receptor protein comprising the amino acid sequence of SEQ ID NO: 56 includes DNA having a nucleotide sequence represented by SEQ ID NO: 57, etc.

The DNA completely coding for the G protein coupled
35 receptor protein of the present invention can be cloned by
(1) carrying out the PCR amplification using a synthetic DNA primer having a partial nucleotide sequence (nucleotide

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fragment) of the G protein coupled receptor protein; or
(2) effecting the selection of a DNA constructed in a
suitable vector, based on the hybridization with a labeled
DNA fragment having part or all of the region encoding a human
5 G protein coupled receptor protein or a labeled synthetic DNA
having part or all of the coding region thereof.

The hybridization is carried out according to methods as
disclosed in, for example, Molecular Cloning, 2nd Ed., J.
Sambrook et al., Cold Spring Harbor Lab. Press, 1989.

10 When a DNA library commercially available in the market is
used, the hybridization is carried out according to protocols
manuals attached thereto.

The cloned G protein coupled receptor protein-
encoding DNA of the present invention can be used as it is, or
15 can be used, as desired, after modifications including
digestion with a restriction enzyme or addition of a linker
or adapter, etc. depending upon objects. The DNA may have
an initiation codon, ATG, on the 5' terminal side and
a termination codon, TAA, TGA or TAG, on the 3' terminal side.
20 These initiation and termination codons can be ligated by
using a suitable synthetic DNA adapter.

An expression vector for G protein coupled receptor
proteins can be produced by, for example, (a) cutting out a
target DNA fragment from the G protein coupled receptor
25 protein-encoding DNA of the present invention and (b) ligating
the target DNA fragment with the downstream site of a promoter
in a suitable expression vector.

The vector may include plasmids derived from
Escherichia coli (e.g., pBR322, pBR325, pUC12, pUC13, etc.),
30 plasmids derived from Bacillus subtilis (e.g., pUB110, pTP5,
pCI94, etc.), plasmids derived from yeasts (e.g., pSH19, pSH15,
etc.), bacteriophages such as λ -phage, and animal virus such
as retrovirus, vaccinia virus and baculovirus.

According to the present invention, any promoter can
35 be used as long as it is compatible with a host which is used
for expressing a gene. When the host for the transformation is
E. coli, the promoters are preferably trp promoters, lac

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promoters, recA promoters, λ_{PL} promoters, lpp promoters, etc. When the host for the transformation is the Bacillus, the promoters are preferably SPO1 promoters, SPO2 promoters, penP promoters, etc. When the host is an yeast, the promoters are 5 preferably PHO5 promoters, PGK promoters, GAP promoters, ADH promoters, etc. When the host is an animal cell, the promoters include SV40-derived promoters, retrovirus promoters, metallothionein promoters, heat shock promoters, cytomegalovirus promoters, SR α promoters, etc. An enhancer 10 can be effectively utilized for the expression.

As required, furthermore, a host-compatible signal sequence is added to the N-terminal side of the G protein coupled receptor protein. When the host is E. coli, the utilizable signal sequences may include alkaline phosphatase 15 signal sequences, OmpA signal sequences, etc. When the host is the Bacillus, they may include α -amylase signal sequences, subtilisin signal sequences, etc. When the host is an yeast, they may include mating factor α signal sequences, invertase signal sequences, etc. When the host is an animal cell, they 20 may include insulin signal sequences, α -interferon signal sequences, antibody molecule signal sequences, etc.

A transformant or transfectant is produced by using the vector thus constructed, which carries the G protein coupled receptor protein-encoding DNA of the present invention. 25 The host may be, for example, Escherichia microorganisms, Bacillus microorganisms, yeasts, insect cells, animal cells, etc. Examples of the Escherichia and Bacillus microorganisms include Escherichia coli K12-DH1 [Proc. Natl. Acad. Sci. USA, Vol. 60, 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, 30 309 (1981)], JA221 [Journal of Molecular Biology, Vol. 120, 517 (1978)], HB101 [Journal of Molecular Biology, Vol. 41, 459 (1969)], C600 [Genetics, Vol. 39, 440 (1954)], etc. Examples of the Bacillus microorganism are, for example, 35 Bacillus subtilis M1114 [Gene, Vol. 24, 255 (1983)], 207-21 [Journal of Biochemistry, Vol. 95, 87 (1984)], etc. The yeast may be, for example, Saccharomyces cerevisiae AH22, AH22R, NA87-11A, DKD-5D, 20B-12, etc. The insect may include

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a silkworm (Bombyx mori larva), [Maeda et al, Nature, Vol. 315, 592 (1985)] etc. The host animal cell may be, for example, monkey-derived cell line, COS-7, Vero, Chinese hamster ovary cell line (CHO cell), DHFR gene-deficient Chinese hamster cell line (dhfr CHO cell), mouse L cell, murine myeloma cell, human FL cell, etc.

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. Transformation of Escherichia microorganisms can be carried out in accordance with methods as disclosed in, for example, Proc. Natl. Acad. Sci. USA, Vol. 69, 2110 (1972), Gene, Vol. 17, 107 (1982), etc. Transformation of Bacillus microorganisms can be carried out in accordance with methods as disclosed in, for example, Molecular & General Genetics, Vol. 168, 111 (1979), etc. Transformation of the yeast can be carried out in accordance with methods as disclosed in, for example, Proc. Natl. Acad. Sci. USA, Vol. 75, 1929 (1978), etc. The insect cells can be transformed in accordance with methods as disclosed in, for example, Bio/Technology, 6, 47-55, 1988. The animal cells can be transformed by methods as disclosed in, for example, Virology, Vol. 52, 456, 1973, etc. The transformants or transfectants which are transformed with expression vectors containing a G protein coupled receptor protein-encoding DNA are produced according to the aforementioned techniques.

Cultivation of the transformant (transfectant) in which the host is Escherichia or Bacillus microorganism can be carried out suitably in a liquid culture medium. The culture medium may contain carbon sources, nitrogen sources, minerals, etc. necessary for growing the transformant. The carbon source may include glucose, dextrin, soluble starch, sucrose, etc. The nitrogen source may include organic or inorganic substances such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extracts, bean-cakes, potato extracts, etc. Examples of the minerals may include calcium chloride, sodium dihydrogen phosphate, magnesium chloride, etc. It is further allowable to add yeasts, vitamins, growth-

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promoting factors, etc. It is desired that the culture medium is pH from about 5 to about 8.

The Escherichia microorganism culture medium is preferably an M9 medium containing, for example, glucose and casamino acid (Miller, Journal of Experiments in Molecular Genetics), 431-433, Cold Spring Harbor Laboratory, New York, 1972. Depending on necessity, the medium may be supplemented with drugs such as 3β -indolyl acrylic acid in order to improve efficiency of the promoter. In the case of the Escherichia host, the cultivation is carried out usually at about 15 to 43 °C for about 3 to 24 hours. As required, aeration and stirring may be applied. In the case of the Bacillus host, the cultivation is carried out usually at about 30 to 40 °C for about 6 to 24 hours. As required, aeration and stirring may be also applied. In the case of the transformant in which the host is an yeast, the culture medium used may include, for example, a Burkholder minimum medium [Bostian, K.L. et al., Proc. Natl. Acad. Sci. USA, Vol. 77, 4505 (1980)], an SD medium containing 0.5% casamino acid [Bitter, G.A. et al., Proc. Natl. Acad. Sci. USA, Vol. 81, 5330 (1984)], etc. It is preferable that pH of the culture medium is adjusted to be from about 5 to about 8. The cultivation is carried out usually at about 20 to 35 °C for about 24 to 72 hours. As required, aeration and stirring may be applied.

In the case of the transformant in which the host is an insect, the culture medium used may include those obtained by suitably adding additives such as passivated (or immobilized) 10% bovine serum and the like to the Grace's insect medium (Grace, T.C.C., Nature, 195, 788 (1962)). It is preferable that pH of the culture medium is adjusted to be about 6.2 to 6.4. The cultivation is usually carried out at about 27 °C for about 3 to 5 days. As desired, aeration and stirring may be applied. In the case of the transformant in which the host is an animal cell, the culture medium used may include MEM medium [Science, Vol. 122, 501 (1952)], DMEM medium [Virology, Vol. 8, 396 (1959)], RPMI 1640 medium [Journal of the American Medical Association, Vol. 199, 519 (1967)], 199 medium [Proceedings of

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the Society of the Biological Medicine, Vol. 73, 1 (1950)], etc. which are containing, for example, about 5 to 20% of fetal calf serum. It is preferable that the pH is from about 5 to about 8. The cultivation is usually carried out at about 30 to 40 °C for about 15 to 60 hours. As required, aeration and stirring may be applied.

Separation and purification of the G protein coupled receptor protein from the above-mentioned cultures can be carried out according to methods described herein below.

To extract G protein coupled receptor proteins from the cultured microorganisms or cells, the microorganisms or cells are collected by known methods after the cultivation, suspended in a suitable buffer solution, disrupted by ultrasonic waves, lysozyme and/or freezing and thawing, etc. and, then, a crude extract of the G protein coupled receptor protein is obtained by centrifugation or filtration. Other conventional extracting or isolating methods can be applied. The buffer solution may contain a protein-denaturing agent such as urea or guanidine hydrochloride or a surfactant such as Triton X-100 (registered trademark, hereinafter often referred to as "TM").

In case where G protein coupled receptor proteins are secreted into culture media, supernatant liquids are separated from the microorganisms or cells after the cultivation is finished and the resulting supernatant liquid is collected by widely known methods. The culture supernatant liquid and extract containing G protein coupled receptor proteins can be purified by suitable combinations of widely known methods for separation, isolation and purification.

The widely known methods of separation, isolation and purification may include methods which utilizes solubility, such as salting out or sedimentation with solvents methods which utilizes chiefly a difference in the molecular size or weight, such as dialysis, ultrafiltration, gel filtration and SDS-polyacrylamide gel electrophoresis, methods utilizing a difference in the electric charge, such as ion-exchange chromatography, methods utilizing specific

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affinity such as affinity chromatography, methods utilizing a difference in the hydrophobic property, such as inverse-phase high-performance liquid chromatography, and methods utilizing a difference in the isoelectric point such as isoelectric 5 electrophoresis, etc.

In case where the G protein coupled receptor protein thus obtained is in a free form, the free protein can be converted into a salt thereof by known methods or method analogous thereto. In case where the G protein coupled 10 receptor protein thus obtained is in a salt form vice versa, the protein salt can be converted into a free form or into any other salt thereof by known methods or method analogous thereto.

The G protein coupled receptor protein produced by 15 the transformant can be arbitrarily modified or a polypeptide can be partly removed therefrom, by the action of a suitable protein-modifying enzyme before or after the purification. The protein-modifying enzyme may include trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase, etc. 20 The activity of the G protein coupled receptor protein thus formed can be measured by experimenting the coupling (or binding) with a ligand or by enzyme immunoassays (enzyme linked immunoassays) using specific antibodies.

The G protein coupled receptor protein-encoding DNA 25 and the G protein coupled receptor protein of the present invention can be used for:

- ① methods of determining ligands for the G protein coupled receptor protein of the present invention,
- ② obtaining an antibody and an antiserum,
- ③ constructing a system for expressing a recombinant receptor protein,
- ④ developing a receptor-binding assay system using the above developing system and screening pharmaceutical candidate compounds,
- ⑤ designing drugs based upon the comparison with ligands and receptors which have a similar or analogous structure,

- ⑥ preparing a probe in the analysis of genes and preparing a PCR primer, and
- ⑦ gene manipulating therapy.

In particular, it is allowable to screen a G protein coupled receptor agonist or antagonist specific to a warm-blooded animal such as human being by a receptor-binding assay system which uses a system for expressing a recombinant G protein coupled receptor protein of the present invention. The agonist or antagonist thus screened or characterized permits various applications including prevention and/or therapy of a variety of diseases.

Concretely described below are uses of G protein coupled receptor proteins, partial peptide thereof (peptide fragment thereof), G protein coupled receptor protein-encoding DNAs and antibodies against the G protein coupled receptor protein according to the present invention.

As hereunder, more detailed description will be made on the usefulness of the G protein coupled receptor protein-encoding DNA obtained by the screening method for G protein coupled receptor protein-encoding DNAs according to the present invention, the G protein coupled receptor proteins encoded by said DNA, peptide fragments or segments thereof (including partial peptides thereof) or salts thereof (hereinafter, those including their salts, will be referred to as the "G protein coupled receptor protein or a peptide fragment thereof"), cells or cell membrane fractions thereof each containing the recombinant type G protein coupled receptor protein, etc. Their various applications are also disclosed herein below.

(1) Method for Determining Ligands to the G Protein Coupled Receptor Protein

The G protein coupled receptor protein (or the peptide segment thereof) is useful as a reagent for investigating or determining a ligand to said G protein coupled receptor protein.

According to the present invention, methods for determining a ligand to the G protein coupled receptor protein

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which comprises contacting the G protein coupled receptor protein or the peptide segment or fragment thereof with the compound to be tested are provided.

The compound to be tested may include not only known
5 ligands such as angiotensins, bombesins, canavinoinds,
cholecystokinins, glutamine, serotonin, melatonin,
neuropeptides Y, opioids, purine, vasopressins, oxytocins,
VIP (vasoactive intestinal and related peptides),
somatostatins, dopamine, motilins, amylin, bradykinins,
10 CGRP (calcitonin gene related peptides), adrenomedullins,
leukotrienes, pancreastatins, prostaglandins, thromboxanes,
adenosine, adrenaline, α - and β -chemokines (IL-8, GRO α ,
GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14,
MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelins,
15 enterogastrins, histamine, neurotensins, TRH, pancreatic
polypeptides, galanin, modified derivatives thereof, analogues
thereof, family members thereof and the like but also tissue
extracts, cell culture supernatants, etc. of warm-blooded
animals (such as mice, rats, swines, cattle, sheep, monkeys
20 and human being), etc. For example, said tissue extract, said
cell culture supernatant, etc. is added to the G protein
coupled receptor protein for measurement of the cell
stimulating activity, etc. and fractionated by relying on the
measurements whereupon a single ligand can be finally
25 obtained.

In one specific embodiment of the present invention,
said method for determining the ligand includes a method for
determining a compound or a salt thereof capable of
stimulating a target cell which comprises binding said compound
30 with the G protein coupled receptor protein either in the
presence of the G protein coupled receptor protein or the
peptide segment thereof or in a receptor binding assay system
in which the expression system for the recombinant type
receptor protein is constructed and used; and measuring the
35 receptor-mediated cell stimulating activity, etc.
Examples of said cell stimulating activities include promoting
activity or inhibiting activity on biological responses,

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e.g. liberation of arachidonic acid, liberation of acetylcholine, liberation of endocellular Ca^{2+} , production of endocellular cAMP, production of endocellular cGMP, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of endocellular protein, activation of c-fos, lowering in pH, activation of G protein, cell promulgation, etc. Examples of said compound or salt capable of stimulating the cell via binding with the G protein coupled receptor protein include peptides, proteins, nonpeptidic compounds, synthetic compounds, fermented products, etc.

In said method for determining the ligand, the characteristic feature is that when the G protein coupled receptor protein or the peptide segment thereof is contacted with the test compound, for example, the binding amount, the cell stimulating activity, etc. of the test compound to the G protein coupled receptor protein or the peptide segment thereof is measured.

In more specific embodiments of the present invention, said methods for determining the ligand includes:

- ① a method of determining a ligand to a G protein coupled receptor protein, which comprises contacting a labeled test compound with a G protein coupled receptor protein or a peptide segment thereof, and measuring the amount of the labeled test compound binding with said protein or salt thereof or with said peptide fragment or salt thereof;
- ② a method of determining a ligand to a G protein coupled receptor protein, which comprises contacting a labeled test compound with cells containing the G protein coupled receptor protein or the membrane fraction of said cell, and measuring the amount of the labeled test compound binding with said cells or said cell fraction;
- ③ a method of determining a ligand to a G protein coupled receptor protein, which comprises contacting a labeled test compound with the G protein coupled receptor protein expressed on cell membranes by culturing transformants containing the DNA coding for the G protein coupled receptor protein, and measuring the amount of the labeled test compound binding with

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said G protein coupled receptor protein;

④ a method of determining a ligand to a G protein coupled receptor protein, which comprises contacting a test compound with cells containing the G protein coupled receptor protein, and measuring the cell stimulating activity (e.g. promoting or inhibiting activity on biological responses such as liberation of arachidonic acid, liberation of acetylcholine, liberation of endocellular Ca^{2+} , production of endocellular cAMP, production of endocellular cGMP, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of endocellular protein, activation of c-fos, lowering in pH, activation of G protein, cell promulgation, etc.) via the G protein coupled receptor protein; and

⑤ a method of determining a ligand to the G protein coupled receptor protein, which comprises contacting a test compound with the G protein coupled receptor protein expressed on the cell membrane by culturing transformants containing the DNA coding for the G protein coupled receptor protein, and measuring the cell stimulating activity (activity for promoting or inhibiting physiological responses such as liberation of arachidonic acid, liberation of acetylcholine, liberation of endocellular Ca^{2+} , production of endocellular cAMP, production of endocellular cGMP, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of endocellular protein, activation of c-fos, lowering in pH, activation of G protein, cell promulgation, etc.) via the G protein coupled receptor protein.

Described below are specific explanations on the determining method of ligands according to the present invention which are provided only for illustrative purposes.

First, the G protein coupled receptor protein used for the method for determining the ligand may include any material so far as it contains a G protein coupled receptor protein or a peptide fragment or segment thereof (including a partial peptide thereof) or a salt thereof although it is preferable to express a large amount of G protein coupled receptor proteins in animal cells.

In the manufacture of the G protein coupled receptor protein, the above-mentioned method can be used and it may be carried out by expressing said protein encoding DNA in mammalian cells or in insect cells. With respect to the DNA fragment coding for the aimed region, complementary DNA may be used although it is not limited thereto. For example, gene fragments or synthetic DNA may be used as well.

In order to introduce the G protein coupled receptor protein-encoding DNA fragment into host animal cells and to express it efficiently, it is preferred that said DNA fragment is incorporated into the downstream site of polyhedron promoters derived from nuclear polyhedrosis virus belonging to baculovirus, promoters derived from SV40, promoters derived from retrovirus, metallothionein promoters, 15 human heat shock promoters, cytomegalovirus promoters, SR α promoters, etc. Examinations of the quantity and the quality of the expressed receptor can be carried out by methods per se known to those of skill in the art or methods similar thereto. For example, they may be conducted by methods 20 described in publications such as Nambi, P. et al: The Journal of Biochemical Society, vol.267, pages 19555-19559 (1992).

Accordingly, with respect to the determination of the ligand, the material containing a G protein coupled receptor protein or peptide segment thereof may include products containing G protein coupled receptor proteins which are purified by methods per se known to those of skill in the art or methods similar thereto, peptide fragments of said G protein coupled receptor protein, cells containing said G protein coupled receptor protein, membrane fractions of the 30 cell containing said protein, etc.

When the G protein coupled receptor protein-containing cell is used in the determining method of the ligand, said cell may be immobilized with binding agents including glutaraldehyde, formalin, etc. The immobilization 35 may be carried out by methods per se known to those of skill in the art or methods similar thereto.

The G protein coupled receptor protein-

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containing cells are host cells expressing the G protein coupled receptor protein. Examples of said host cells are microorganisms such as Escherichia coli, Bacillus subtilis, yeasts, insect cells, animal cells, etc.

5 The cell membrane fraction is a cell membrane-rich fraction which is prepared by methods per se known to those of skill in the art or methods similar thereto after disruption of cells. Examples of cell disruption may include a method for squeezing cells using a Potter-Elvehjem homogenizer,

10 a disruption by a Waring blender or a Polytron (manufactured by Kinematica), a disruption by ultrasonic waves, a disruption via blowing out cells from small nozzles together with applying a pressure using a French press or the like, etc. In the fractionation of the cell membrane, a fractionation method by

15 means of centrifugal force such as a fractional centrifugal separation and a density gradient centrifugal separation is mainly used. For example, disrupted cellular liquid is centrifuged at a low speed (500 rpm to 3,000 rpm) for a short period (usually, from about one to ten minutes), the

20 supernatant liquid is further centrifuged at a high speed (1,500 rpm to 3,000 rpm) usually for 30 minutes to two hours and the resulting precipitate is used as a membrane fraction. Said membrane fraction contains a lot of the expressed G protein coupled receptor protein and a lot of membrane

25 components such as phospholipids and membrane proteins derived from the cells.

The amount of the G protein coupled receptor protein in the membrane fraction cell containing said G protein coupled receptor protein is preferably 10^3 - 10^8 molecules per cell or, suitably, 10^5 to 10^7 molecules per cell.

30 Incidentally, the more the expressed amount, the higher the ligand binding activity (specific activity) per membrane fraction whereby the construction of a highly sensitive screening system becomes possible and, moreover, it may enable us to measure the large amount of samples within the same lot.

35 In conducting the above-mentioned methods ① to ② wherein ligands capable of binding with the G protein coupled

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receptor protein are determined, a suitable G protein coupled receptor fraction and a labeled test compound are necessary. The G protein coupled receptor fraction is preferably a naturally occurring (natural type) G protein coupled receptor, 5 a recombinant type G protein coupled receptor having the activity equivalent to that of the natural type. Here, the term "activity equivalent to" means the equivalent ligand binding activity, etc.

Suitable examples of the labeled test compound are
10 angiotensin, bombesin, canavinoind, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive intestinal and related peptides), somatostatin, dopamine, motilin, amylin, bradykinin, CGRP (calcitonin gene related peptides), adrenomedullin,
15 leukotriene, pancreastatin, prostaglandin, thromboxane, adenosine, adrenaline, α - and β -chemokine (IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neurotensin, TRH, pancreatic
20 polypeptides, galanin, an analogue derivative thereof, etc. which are labeled with [3 H], [125 I], [14 C], [35 S], etc.

Specifically, the determination of ligands capable of binding with G protein coupled receptor proteins is carried out as follows:

25 First, cells or cell membrane fractions containing the G protein coupled receptor protein are suspended in a buffer suitable for the determining method to prepare the receptor sample in conducting the method of determining the ligand binding with the G protein coupled receptor protein.
30 The buffer may include any buffer such as Tris-HCl buffer or phosphate buffer with pH 4-10 (preferably, pH 6-8), etc., as long as it does not inhibit the binding of the ligand with the receptor. In addition, surface-active agents such as CHAPS, Tween 80TM (Kao-Atlas, Japan), digitonin, deoxycholate,
35 etc. and various proteins such as bovine serum albumin (BSA), gelatin, milk derivatives, etc. may be added to the buffer with an object of decreasing the non-specific binding.

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Further, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by Peptide Laboratory), pepstatin, etc. may be added with an object of inhibiting the decomposition of the receptor and the ligand by protease. A test compound labeled 5 with a predetermined (or certain) amount (5,000 cpm to 500,000 cpm) of [³H], [¹²⁵I], [¹⁴C], [³⁵S], etc. is made copresent in 0.01 ml to 10 ml of said receptor solution.

In order to know the non-specific binding amount (NSB), a reaction tube to which a great excessive amount of the 10 unlabeled test compound is added is prepared as well. The reaction is carried out at 0-50°C (preferably at 4-37°C) for 20 minutes to 24 hours (preferably 30 minutes to three hours). After the reaction, it is filtered through a glass fiber filter or the like, washed with a suitable amount of 15 the same buffer and the radioactivity remaining in the glass fiber filter is measured by means of a liquid scintillation counter or a gamma-counter. The test compound in which the count (B - NSB) obtained by subtracting the non-specific binding amount (NSB) from the total binding amount (B) is 20 more than 0 cpm can be selected as a ligand to the G protein coupled receptor protein of the present invention.

In conducting the above-mentioned methods ④ to ⑥ wherein ligands capable of binding with the G protein coupled receptor protein are determined, the cell stimulating activity 25 (e.g. the liberation of arachidonic acid, the liberation of acetylcholine, endocellular Ca ²⁺ liberation, endocellular cAMP production, the production of insitol phosphate, changes in the cell membrane potential, the phosphorylation of endocellular protein, the activation of c-fos, lowering of pH, 30 the activation of G protein, cell promulgation, etc.) mediated by the G protein coupled receptor protein may be measured by known methods or by the use of commercially available measuring kits. To be more specific, G protein coupled receptor protein-containing cells are at first cultured in a 35 multi-well plate or the like.

In conducting the determination of ligand, it is substituted with a fresh medium or a suitable buffer which

does not show toxicity to the cells in advance of the experiment, and incubated for certain period after adding a test compound, etc. thereto. Then, the cells are extracted or the supernatant liquid is recovered and the resulting product 5 is determined by each of the methods. When it is difficult to identify the production of the substance (e.g. arachdonic acid) which is to be an index for the cell stimulating activity due to the decomposing enzyme contained in the cell, an assay may be carried out by adding an inhibitor against said decomposing 10 enzyme. With respect to the activity such as an inhibitory action against cAMP production, it may be detected as an inhibitory action against the production of the cells whose fundamental production is increased by forskolin or the like.

The kit used for the method of determining the ligand binding with the G protein coupled receptor protein includes a G protein coupled receptor protein or a peptide fragment thereof, cells containing the G protein coupled receptor protein, a membrane fraction from the cells containing the G protein coupled receptor protein, etc.

20 Examples of the kit for determining the ligand are as follows:

1. Reagent for Determining the Ligand.

① Buffer for Measurement and Buffer for Washing.

25 The buffering product wherein 0.05% of bovine serum albumin (manufactured by Sigma) is added to Hanks' Balanced Salt Solution (manufactured by Gibco).

This product may be sterilized by filtration through a membrane filter with a 0.45 μ m pore size, and stored at 4°C or may be formulated upon use.

30 ② G Protein Coupled Receptor Protein Sample.

CHO cells in which G protein coupled receptor proteins are expressed are subcultured at the rate of 5×10^5 5 cells/well in a 12-well plate and cultured at 37°C in a humidified 5% CO₂/95% air atmosphere for two days to prepare the sample.

③ Labeled Test Compound.

The compound which is labeled with commercially

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available [³H], [¹²⁵I], [¹⁴C], [³⁵S], etc. or labeled with a suitable method.

5 The product in a state of an aqueous solution is stored at 4°C or at -20°C and, upon use, diluted to 1 μM with a buffer for the measurement. In the case of the test compound which is hardly soluble in water, it is dissolved in dimethylformamide, DMSO, methanol, etc.

④ Unlabeled Test Compound.

10 The same compound for the labeled one is prepared in a concentration of 100 to 1,000-fold concentrated state.

2. Method of Measurement.

15 ① G protein coupled receptor protein-expressing CHO cells cultured in a 12-well tissue culture plate are washed twice with 1 ml of buffer for the measurement and then 490 μ l of buffer for the measurement is added to each well.

② Five μ l of the labeled test compound is added and the mixture is made to react at room temperature for one hour. For measuring the nonspecific binding amount, 5 μ l of the unlabeled test compound is added.

20 ③ The reaction solution is removed from each well, which is washed with 1 ml of a buffer for the measurement three times. The labeled test compound which is binding with the cells is dissolved in 0.2N NaOH-1% SDS and mixed with 4 ml of a liquid scintillator A (manufactured by Wako Pure Chemical, Japan).

25 ④ Radioactivity is measured using a liquid scintillation counter (manufactured by Beckmann).

30 The ligand which can bind with the G protein coupled receptor protein include substances occurring or existing, for example, in brain, pituitary gland, pancreas, etc. Examples of the ligand are angiotensin, bombesin, canavinoind, cholecystokinin, glutamine, serotonin, melatonin, neuropeptide Y, opioid, purine, vasopressin, oxytocin, VIP (vasoactive intestinal and related peptide), somatostatin, dopamine, 35 motilin, amylin, bradykinin, CGRP (calcitonin gene related

peptide), adrenomedullin, leukotriene, pancreastatin, prostaglandin, thromboxane, thromboxatin, adenosine, adrenaline, α - and β -chemokine (IL-8, GRO α , GRO β , GRO γ , NAP-2, ENA-78, PF4, IP10, GCP-2, MCP-1, HC14, MCP-3, I-309, 5 MIP1 α , MIP-1 β , RANTES, etc.), endothelin, enterogastrin, histamine, neuropeptides, TRH, pancreatic polypeptide, galanin, modified derivatives thereof, analogues thereof, etc.

Since the receptor protein encoded by pMAH2-17 is highly homologous to purinoceptors, it is considered that there 10 are strong possibility of a subtype within purinoceptor families. All data including electrophysiological measurements are supporting that the mouse pancreatic β -cell strain, MIN6-derived receptor protein of the present invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by 15 pMAH2-17) is a novel purinoceptor subtype. In other words, it is suggested that the ligand capable of binding with the mouse pancreatic β -cell strain, MIN6-derived receptor protein of the present invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by pMAH2-17) is a purine 20 compound such as ATP. Further, the receptor protein (e.g., SEQ ID NO: 56, or proteins encoded by phAH2-17) is considered to be a novel human type purinoceptor. It is presumed that it is advantageously useful in efficiently screening for agonists or antagonists to receptor proteins which control or regulate 25 functions in the central nervous system or immune system, related to purine compounds, and in developing pharmaceuticals.

(2) Preventive and Therapeutic Agent for of G Protein
Conjugated Receptor Protein Deficiency Diseases

If a ligand to the G protein coupled receptor protein 30 is disclosed via the aforementioned method (1), the G protein coupled receptor protein-encoding DNA can be used a preventive and/or therapeutic agent for treating said G protein coupled receptor protein deficiency diseases depending upon the action that said ligand exerts.

35 For example, when there is a patient for whom the physiological action of the ligand cannot be expected because

of a decrease in the G protein coupled receptor protein in vivo, the amount of the G protein coupled receptor protein in the brain cells of said patient can be increased whereby the action of the ligand can be fully achieved by:

5 (a) administering the G protein coupled receptor protein-encoding DNA to the patient to express it; or
(b) inserting the G protein coupled receptor protein-encoding DNA into brain cells or the like to express it, followed by transplanting said brain cells or the like to said patient.

10 Accordingly, the G protein coupled receptor protein-encoding DNA can be used as a safe and less toxic preventive and therapeutic agent for the G protein coupled receptor protein deficiency diseases. In an embodiment, it is suggested that the ligands capable of binding with the mouse pancreatic

15 β -cell strain, MIN6-derived receptor protein of the present invention (e.g., SEQ ID NO: 38 and SEQ ID NO: 39, or proteins encoded by pMAH2-17) and further with the human-derived receptor protein of the present invention (e.g., SEQ ID NO: 56, or proteins encoded by phAH2-17) are purine compounds such as
20 ATP. Therefore, the disease to be treated may include diseases or syndromes in connection with purine ligand compounds.

Examples of such diseases may include cancer, immunodeficiency, autoimmune disease, rheumatoid arthritis, rejection of internal organ transplant, hypertension, diabetes, cystic fibrosis, hypotension, incontinence of urine, pain, etc.

(3) Preventive and Therapeutic Pharmaceutical Composition for Human-Derived G Protein Conjugated Receptor Protein Deficiency Diseases

If the human-derived G protein coupled receptor protein-encoding DNA is screened and a ligand for said human-derived G protein coupled receptor protein can be clarified using the above-mentioned method (1), the human-derived G protein coupled receptor protein-encoding DNA can be used as an agent for the prevention or therapy of the deficiency diseases of said human-derived G protein coupled receptor protein depending upon the action that said ligand exhibits.

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For example, when there is a patient for whom the physiological action of the ligand cannot be expected because of a decrease in the G protein coupled receptor protein in vivo, the amount of the G protein coupled receptor protein in the 5 brain cells of said patient can be increased whereby the action of the ligand can be fully achieved by:

(a) administering the G protein coupled receptor protein-encoding DNA to the patient to express it; or

(b) inserting the G protein coupled receptor protein-encoding 10 DNA into brain cells or the like to express it, followed by transplanting said brain cells or the like to said patient. Accordingly, the G protein coupled receptor protein-encoding DNA can be used as a safe and less toxic preventive and therapeutic agent for the G protein coupled receptor protein 15 deficiency diseases.

When the G protein coupled receptor protein-encoding DNA is used as the above-mentioned agent, said DNA may be used alone or after inserting it into a suitable vector such as retrovirus vector, adenovirus vector, adenovirus-associated 20 virus vector, etc. followed by subjecting the product vector to a conventional means. Thus, it may be administered orally parenterally, by inhalation spray, rectally, or topically as pharmaceutical compositions or formulations. Oral formulations include tablets (sugar-coated if necessary), capsules, 25 elixirs, microcapsules, etc. Parenteral formulations include injections such as an aseptic solution or a suspension in water or in other pharmaceutically acceptable liquid. For example, the DNA of the present invention is admixed in a unit dose form which is required for preparing generally approved 30 pharmaceutical preparations together with a physiologically acceptable carriers, flavoring agents, adjuvants, excipients, diluents, fillers, vehicles, antiseptics, stabilizers, binders, etc. whereupon the preparation can be manufactured. The amount of the effective component in those preparations is to be in 35 such an extent that the suitable dose within an indicated range is achieved.

Examples of the additives which can be admixed in the

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tablets, capsules, etc. are binders such as gelatin, corn starch, tragacanth and gum arabicum; fillers such as crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricating agents such as magnesium stearate; sweetening agents such as sucrose, lactose and saccharine; and flavoring agents such as pepper mint, akamono oil and cherry. When the unit dose form of the preparation is a capsule, a liquid carrier such as fat/oil may be further added in addition of the above-mentioned types of materials.

5 The aseptic composition for injection may be formulated by conventional practices for the preparations such as that the active substance in a vehicle such as water for injection is dissolved or suspended in naturally occurring plant oil such as sesame oil and palm oil.

10 Examples of an aqueous liquid for injection are a physiological saline solution and isotonic solutions containing glucose and other auxiliary agents (e.g. D-sorbitol, D-mannitol, sodium chloride, etc.) wherein a suitable auxiliary solubilizers such as alcohol (e.g. ethanol, etc.), polyalcohol (e.g.

15 propylene glycol polyethylene glycol, etc.), nonionic surface-active agent (e.g. Polysorbate 80TM, HCO-50, etc.), etc. may be jointly used. Examples of an oily liquid include sesame oil, soybean oil, etc. wherein benzyl benzoate, benzyl alcohol, etc. may be jointly used as auxiliary solubilizers. In addition,

20 buffers (e.g. phosphate buffer, sodium acetate buffer, etc.), analgesic agents (e.g. benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g. human serum albumin, polyethylene glycol, etc.), stabilizers (e.g. benzyl alcohol phenol, etc.), antioxidants, etc. may be admixed therewith too.

25 The prepared injection solution is filled in suitable ampoules. The preparation prepared as such is safe and less toxic and, therefore, it can be administered to warm-blooded animals (e.g., rat, rabbit, sheep, swine, cattle, cat, dog, monkey, human beings, etc.).

30 Specific dose levels of said DNA may vary depending upon a variety of factors including the activity of drugs employed, the age, body weight, general health, sex, diet,

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time of administration, route of administration, drug combination, and the severity of the symptom. In the case of oral administration, it is usually about 0.1-100 mg, preferably about 1.0-50 mg or, more preferably, about 1.0-20 mg per day
5 for adults (as 60 kg). When it is administered parenterally, its dose at a time may vary depending upon the object (patient) to be administered, organs to be administered, symptoms, administering methods, etc. but, in the case of injections, it is usually convenient to give by an intravenous route in an
10 amount of about 0.01-30 mg, preferably about 0.1-20 mg or, more preferably, about 0.1-10mg per day to adults (as 60 kg).
In the case of other animals, the dose calculated for 60 kg may be administered as well.

(4) Quantitative Determination of Ligand to the G Protein
15 Conjugated Receptor Protein of the Present Invention.

The G protein coupled receptor protein or a peptide fragment thereof has a binding property to ligand and, therefore, it is capable of determining quantitatively an amount of ligands in vivo with good sensitivity.

20 This quantitative determination may be carried out by, for example, combining with a competitive method. Thus, samples to be determined is contacted with G protein coupled receptor proteins or peptide fragments thereof so that the ligand concentration in said sample can be determined.

25 In one embodiment of the quantitative determination, the protocols described in the following ① and ② or the methods similar thereto may be used:

- ① Hiroshi Irie (ed): "Radioimmunoassay" (Kodansha, Japan, 1974); and
- 30 ② Hiroshi Irie (ed): "Radioimmunoassay, Second Series" (Kodansha, Japan, 1979).

(5) Screening of Compound Inhibiting the Binding of Ligand with the G Protein Conjugated Receptor Protein of the Present Invention.

35 G Protein coupled receptor proteins or peptide

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fragments thereof are used. Alternatively, expression systems for recombinant type G Protein coupled receptor proteins or peptide fragments thereof are constructed and receptor binding assay systems using said expression system are used. In these 5 assay systems, it is possible to screen compounds (e.g. peptides, proteins, nonpeptidic compounds, synthetic compounds, fermented products, cell extracts, plant extracts, animal tissue extracts, etc.) or salts thereof which inhibits the binding of a ligand with the G protein coupled receptor 10 protein. Such a compound includes a compound exhibiting a G protein coupled receptor-mediated cell stimulating activity (e.g. activity of promoting or activity of inhibiting physiological reactions including liberation of arachdonic acid, liberation of acetylcholine, endocellular Ca^{2+} liberation, 15 endocellular cAMP production, endocellular cGMP production, production of inositol phosphate, changes in cell membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein, cell promulgation, etc.) (so-called "G protein coupled receptor-agonist"), a compound free of such a cell stimulating 20 activity (so-called "G protein coupled receptor-antagonist"), etc.

Thus, the present invention provides a method of screening a compound which inhibits the binding of a ligand with a G protein coupled receptor protein or a salt thereof, characterized in comparing the following two cases:
(i) the case wherein the ligand is contacted with the G protein coupled receptor protein or salt thereof, or a peptide fragment thereof or a salt thereof; and
30 (ii) the case wherein the ligand is contacted with a mixture of the G protein coupled receptor protein or salt thereof or the peptide fragment or salt thereof and said test compound.

In said screening method, one characteristic feature of the present invention resides in that the amount of the 35 ligand bonded with said G protein coupled receptor protein or the peptide fragment thereof, the cell stimulating activity of the ligand, etc. are measured in the case where (i) the

ligand is contacted with G protein coupled receptor proteins or peptide fragments thereof and in the case where (ii) the ligand and the test compound are contacted with the G protein coupled receptor protein or the peptide fragment thereof, respectively
5 and then compared therebetween.

In one more specific embodiment of the present invention, the following is provided:

- ① a method of screening a compound or a salt thereof which inhibits the binding of a ligand with a G protein coupled receptor protein, characterized in that, when a labeled ligand is contacted with a G protein coupled receptor protein or a peptide fragment thereof and when a labeled ligand and a test compound are contacted with a G protein coupled receptor protein or a peptide fragment thereof, the amounts of the labeled ligand bonded with said protein or peptide fragment thereof or salt thereof are measured and compared;
- ② a method of screening a compound or a salt thereof which inhibits the binding of a ligand with a G protein coupled receptor protein, characterized in that, when a labeled ligand is contacted with cells containing G protein coupled receptor proteins or a membrane fraction of said cells and when a labeled ligand and a test compound are contacted with cells containing G protein coupled receptor proteins or a membrane fraction of said cells, the amounts of the labeled ligand binding with said protein or peptide fragment thereof or salt thereof are measured and compared;
- ③ a method of screening a compound or a salt thereof which inhibits the binding of a ligand with a G protein coupled receptor protein, characterized in that, when a labeled ligand is contacted with G protein coupled receptor proteins expressed on the cell membrane by culturing a transformant containing a G protein coupled receptor protein encoding DNA and when a labeled ligand and a test compound are contacted with G protein coupled receptor proteins expressed on the cell membrane by culturing a transformant containing a G protein coupled receptor protein encoding DNA, the amounts of the labeled ligand binding with said G protein coupled receptor

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protein are measured and compared;

④ a method of screening a compound or a salt thereof which inhibits the binding of a ligand with a G protein coupled receptor protein, characterized in that, when a G protein

5 coupled receptor protein-activating compound (e.g. a ligand to the G protein coupled receptor protein) is contacted with cells containing G protein coupled receptor proteins and when the G protein coupled receptor protein-activating compound and a test compound are contacted with cells containing G
10 protein coupled receptor proteins, the resulting G protein coupled receptor protein-mediated cell stimulating activities (e.g. activities of promoting or activities of inhibiting physiological responses including liberation of arachdonic acid, liberation of acetylcholine, endocellular Ca^{2+} liberation,
15 endocellular cAMP production, endocellular cGMP production, production of inositol phosphate, changes in cell membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein, cell promulgation, etc.) are measured and compared; and

20 ⑤ a method of screening a compound or a salt thereof which inhibits the binding of a ligand with a G protein coupled receptor protein, characterized in that, when a G protein coupled receptor protein-activating compound (e.g. a ligand to the G protein coupled receptor protein) is contacted with G

25 protein coupled receptor proteins expressed on cell membranes by culturing transformants containing G protein coupled receptor protein-encoding DNA and when a G protein coupled receptor protein-activating compound and a test compound are contacted with the G protein coupled receptor protein
30 expressed on the cell membrane by culturing the transformant containing the G protein coupled receptor protein-encoding DNA, the resulting G protein coupled receptor protein-mediated cell stimulating activities (activities of promoting or activities of inhibiting physiological responses such as liberation of arachdonic acid, liberation of acetylcholine, endocellular Ca^{2+}
35 liberation, endocellular cAMP production, endocellular cGMP production, production of inositol phosphate, changes in cell

- 1 5 8 -

membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein, and cell promulgation) are measured and compared.

Before the G protein coupled receptor protein of the present invention was obtained, the G protein coupled receptor agonist or antagonist had to be screened by, first, obtaining a candidate compound by using G protein coupled receptor protein-containing cells, tissues or cell membrane fractions derived from rat or the like (primary screening) and, then, making sure whether the candidate compound really inhibits the binding between human G protein coupled receptor proteins and ligands (secondary screening). Other receptor proteins inevitably exist when the cells, the tissues or the cell membrane fractions are used as they are, whereby they intrinsically make it difficult to screen agonists or antagonists to the desired receptor proteins. By using the human-derived G protein coupled receptor protein, however, there is no need of effecting the primary screening, whereby it is allowable to efficiently screen a compound that inhibits the binding between a ligand and a G protein coupled receptor. Besides, it is allowable to evaluate whether the compound that is screened is a G protein coupled receptor agonist or a G protein coupled receptor antagonist.

Specific explanations of the screening method will be given as hereunder.

First, with respect to the G protein coupled receptor protein used for the screening method of the present invention, any product may be used so far as it contains G protein coupled receptor proteins or peptide fragment thereof although the use of a membrane fraction of mammalian organs is suitable. However, human organs is extremely hardly available and, accordingly, G protein coupled receptor proteins which are expressed in a large amount using a recombinant are suitable for the screening.

In the manufacture of the G protein coupled receptor protein, the above-mentioned method can be used and it may be carried out by expressing the DNA coding for said protein in

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mammalian cells or in insect cells. With respect to the DNA fragment coding for the target region, complementary DNA may be used although it is not limited thereto. Thus, for example, gene fragments or synthetic DNA may be used as well.

5 In order to introduce the G protein coupled receptor protein-encoding DNA fragment into host animal cells and to express it efficiently, it is preferred that said DNA fragment is incorporated into the downstream of polyhedron promoter of nuclear polyhedrosis virus belonging to baculovirus, promoter 10 derived from SV40, promoter of retrovirus, metallothionein promoter, human heat shock promoter, cytomegalovirus promoter, SRA promoter, etc. Examinations of the quantity and the quality of expressed receptors can be carried out by known methods per se or modified methods substantially analogous 15 thereto. For example, they may be conducted by the method described in publications such as Nambi, P. et al.: The Journal of Biochemical Society, vol.267, pages 19555-19559 (1992).

20 Accordingly, in the screening method, the substance containing a G protein coupled receptor protein or a peptide fragment thereof may be a G protein coupled receptor protein which is purified by known methods per se or a G protein coupled receptor protein fragment which is purified by known methods per se, or a cell containing said protein or a cell 25 membrane fraction of the cell containing said protein, etc.

When the G protein coupled receptor protein-containing cells are used in the screening method, said cells may be immobilized with glutaraldehyde, formalin, etc. The immobilization may be carried out by known methods per se 30 or modified methods substantially analogous thereto.

The G protein coupled receptor protein-containing cells are host cells expressing the G protein coupled receptor protein. Examples of said host cells may include Escherichia coli, Bacillus subtilis, yeasts, insect cells, 35 animal cells such as CHO cell and COS cell, etc.

Cell membrane fractions are fractions which contain a lot of cell membranes prepared by known methods per se or

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modified methods substantially analogous thereto after disrupting or crushing the cells. Examples of disruptions of the cell may include methods by squeezing the cells with a Potter-Elvejem homogenizer, disrupting or crushing by a Waring 5 blender or a Polytron (manufactured by Kinematica), disrupting or crushing by means of ultrasonic wave, disrupting by blowing out the cells from small nozzles together with applying a pressure with a French press or the like, etc.

Fractionation of the cell membrane is carried out mainly by 10 fractionation techniques by means of centrifugal force such as a fractional centrifugal separation and a density gradient centrifugal separation. For example, disrupted liquid of cells is centrifuged at a low speed (500 rpm to 3,000 rpm) for a short period (usually, from about one to ten minutes), the 15 supernatant liquid is further centrifuged at a high speed (1,500 rpm to 3,000 rpm) usually for 30 minutes to two hours and the resulting precipitate is used as a membrane fraction. Said membrane fraction contains a lot of expressed G protein coupled receptor proteins and membrane components such as phospholipids and membrane proteins derived from the cells.

The amount of the G protein coupled receptor protein in the G protein coupled receptor protein-containing cell and in the cell membrane fraction obtained from the cell is preferably 10^3 - 10^8 molecules per cell or, suitably, 25 10^5 to 10^7 molecules per cell. Incidentally, the more the expressed amount, the higher the ligand binding activity (specific activity) per membrane fraction whereby the construction of a highly sensitive screening system is possible and, moreover, it is possible to measure the large 30 amount of samples in the same lot.

In conducting the above-mentioned methods ① to ③ for screening the compound capable of inhibiting the binding of the ligand with the G protein coupled receptor protein, a suitable G protein coupled receptor fraction and a labeled 35 ligand are necessary. With respect to the G protein coupled receptor fraction, it is preferred to use naturally occurring G protein coupled receptors (natural type G protein coupled

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receptors) or recombinant type G protein coupled receptor fractions with the activity equivalent to that of the natural type G protein coupled. Here the term "activity equivalent to" means the same ligand binding activity, or the substantially equivalent ligand binding activity.

With respect to the labeled ligand, it is possible to use labeled ligands, labeled ligand analogized compounds, etc. For example, ligands labeled with [³H], [¹²⁵I], [¹⁴C], [³⁵S], etc. and other labeled substances may be utilized.

Specifically, G protein coupled receptor protein-containing cells or cell membrane fractions are first suspended in a buffer which is suitable for the determining method to prepare the receptor sample in conducting the screening for a compound which inhibits the binding of the ligand with the G protein coupled receptor protein.

With respect to the buffer, any buffer such as Tris-HCl buffer or phosphate buffer of pH 4-10 (preferably, pH 6-8) which does not inhibit the binding of the ligand with the receptor may be used.

In addition, a surface-active agent such as CHAPS, Tween 80TM (Kao-Atlas, Japan), digitonin, deoxycholate, etc. and/or various proteins such as bovine serum albumin (BSA), gelatine, etc. may be added to the buffer with an object of decreasing the nonspecific binding. Further, a protease inhibitor such as PMSF, leupeptin, E-64 (manufactured by Peptide Laboratory, Japan), pepstatin, etc. may be added with an object of inhibiting the decomposition of the receptor and the ligand by protease. A labeled ligand in a certain amount (5,000 cpm to 500,000 cpm) is added to 0.01 ml to 10 ml of said receptor solution and, at the same time, 10⁻⁴ M to 10⁻¹⁰ M of a test compound is made copresent. In order to determine the nonspecific binding amount (NSB), a reaction tube to which a great excessive amount of unlabeled test compounds is added is prepared as well.

The reaction is carried out at 0-50°C (preferably at 4-37°C) for 20 minutes to 24 hours (preferably 30 minutes to three hours). After the reaction, it is filtered through a

glass fiber filter, a filter paper, or the like, washed with a suitable amount of the same buffer and the radioactivity retained in the glass fiber filter, etc. is measured by means of a liquid scintillation counter or a gamma-counter.

5 Supposing that the count (B_0 - NSB) obtained by subtracting the nonspecific binding amount (NSB) from the total binding amount (B_0) wherein an antagonizing substance is not present is set at 100%, the test compound in which the specific binding amount (B - NSB) obtained by subtracting the nonspecific binding
10 amount (NSB) from the total binding amount (B) is, for example, less than 50% may be selected as a candidate ligand to the G protein coupled receptor protein of the present invention.

In conducting the above-mentioned methods ④ to ⑤ for screening the compound which inhibits the binding of the
15 ligand with the G protein coupled receptor protein, the G protein coupled receptor protein-mediated cell stimulating activity (e.g. activities of promoting or activities of inhibiting physiological responses such as liberation of arachidonic acid, liberation of acetylcholine, endocellular
20 Ca^{2+} liberation, endocellular cAMP production, production of inositol phosphate, changes in the cell membrane potential, phosphorylation of endocellular proteins, activation of c-fos, lowering of pH, activation of G protein and cell promulgation, etc.) may be measured by known methods or by the use of
25 commercially available measuring kits. To be more specific, G protein coupled receptor protein-containing cells are at first cultured in a multiwell plate or the like.

In conducting the screening, it is substituted with a suitable buffer which does not show toxicity to fresh media
30 or cells in advance, incubated for a certain period after adding a test compound, etc. thereto. The resultant cells are extracted or the supernatant liquid is recovered and the resulting product is determined, preferably quantitatively, by each of the methods. When it is difficult to identify the
35 production of the index substance (e.g. arachidonic acid, etc.) which is to be an index for the cell stimulating activity due to the presence of decomposing enzymes contained in the cell,

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an assay may be carried out by adding an inhibitor against said decomposing enzyme. With respect to the activities such as an inhibitory action against cAMP production, it may be detected as an inhibitory action against the cAMP production
5 in the cells whose fundamental production has been increased by forskolin or the like.

In conducting a screening by measuring the cell stimulating activity, cells in which a suitable G protein coupled receptor protein is expressed are necessary.

10 Preferred G protein coupled receptor protein-expressing cells are naturally occurring G protein coupled receptor protein (natural type G protein coupled receptor protein)-containing cell lines or strains (e.g. mouse pancreatic β cell line, MIN6, etc.), the above-mentioned recombinant type G protein
15 coupled receptor protein-expressing cell lines or strains, etc.

Examples of the test compound includes peptides, proteins, non-peptidic compounds, synthesized compounds, fermented products, cell extracts, plant extracts, animal
20 tissue extracts, serum, blood, body fluid, etc. Those compounds may be novel or known.

A kit for screening the compound which inhibits the binding of the ligand with the G protein coupled receptor protein or a salt thereof of the present invention comprises
25 a G protein coupled receptor protein or a peptide fragment thereof, or G protein coupled receptor protein-containing cells or cell membrane fraction thereof.

Examples of the screening kit include as follows:

1. Reagent for Determining Ligand.

30 ① Buffer for Measurement and Buffer for Washing.

The product wherein 0.05% of bovine serum albumin (manufactured by Sigma) is added to Hanks' Balanced Salt Solution (manufactured by Gibco).

This may be sterilized by filtration through
35 a membrane filter with a 0.45 μ m pore size, and stored at 4°C or may be prepared upon use.

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② Sample of G Protein Conjugated Receptor Protein.

CHO cells in which a G protein coupled receptor protein is expressed are subcultured at the rate of 5×10^5 cells/well in a 12-well plate and cultured at 37°C with a 5% CO₂ and 95% air atmosphere for two days to prepare the sample.

③ Labeled Ligand.

The ligand which is labeled with commercially available [³H], [¹²⁵I], [¹⁴C], [³⁵S], etc.

10 The product in a state of an aqueous solution is stored at 4°C or at -20°C and, upon use, diluted to 1 μM with a buffer for the measurement.

④ Standard Ligand Solution.

15 Ligand is dissolved in PBS containing 0.1% of bovine serum albumin (manufactured by Sigma) to make 1 mM and stored at -20°C.

2. Method of the Measurement.

① CHO cells are cultured in a 12-well tissue culture plate to express G protein coupled receptor proteins. The G protein coupled receptor protein-expressing CHO cells are washed with 20 1 ml of buffer for the measurement twice. Then 490 μl of buffer for the measurement is added to each well.

② Five μl of a test compound solution of 10^{-3} to 10^{-10} M is added, then 5 μl of a labeled ligand is added and is made to react at room temperature for one hour. For knowing 25 the non-specific binding amount, 5 μl of the ligand of 10^{-3} M is added instead of the test compound.

③ The reaction solution is removed from the well, which is washed with 1 ml of buffer for the measurement three times. The labeled ligand binding with the cells is dissolved in 0.2N 30 NaOH-1% SDS and mixed with 4 ml of a liquid scintillator A (manufactured by Wako Pure Chemical, Japan).

④ Radioactivity is measured using a liquid scintillation counter (manufactured by Beckmann) and PMB (percent of maximum binding) is calculated by the following expression:

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PMB: Percent of maximum binding
B: Value when a sample is added
NSB: Nonspecific binding
 B_0 : Maximum binding

5 The compound or a salt thereof obtained by the screening method or by the screening kit is a compound which inhibits the binding of a ligand with a G protein coupled receptor protein and, more particularly, it is a compound having a cell stimulating activity mediated via a G protein
10 coupled receptor or a salt thereof (so-called "G protein coupled receptor agonist") or a compound having no said stimulating activity (so-called "G protein coupled receptor antagonist"). Examples of said compound are peptides, proteins, non-peptidic compounds, synthesized compounds, fermented products, etc. and the compound may be novel or known.
15

Said G protein coupled receptor agonist has the same physiological action as the ligand to the G protein coupled receptor protein has and, therefore, it is useful as a safe and less toxic pharmaceutical composition depending upon said ligand activity.

On the other hand, said G protein coupled receptor antagonist is capable of inhibiting the physiological activity of the ligand to the G protein coupled receptor protein and, therefore, it is useful as a safe and less toxic pharmaceutical composition for inhibiting said ligand activity.

It is also strongly suggested that agonists and/or antagonists related to the receptor encoded by pMAH2-17 obtained in Example 19 and/or the receptor encoded by phAH2-17 obtained in Example 21 would be useful in therapeutic or prophylactic treatment of diseases or syndromes in connection with purine ligand compounds or related analogues. It is expected that the agonists of the receptor encoded by pMAH2-17 and/or of the receptor encoded by phAH2-17 are useful as an immunomodulator or an antitumor agent, in addition they are

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useful in therapeutically or prophylactically treating hypertension, diabetes, cystic fibrosis, etc. It is still expected that the antagonists of the receptor encoded by pMAH2-17 and/or of the receptor encoded by phAH2-17 are useful
5 as hypotensive agents, analgesics, agents for therapeutically or prophylactically treating incontinence of urine, etc. With regard to purinoceptors, the mutation of conserved basic amino acid residues in the 6th or 7th putative transmembrane domain of purinoceptors introduces alteration into the receptor's
10 responses to ATP (J. Biol. Chem., Vol. 270(9), pp. 4185-4188 (1995)). It is suggested that ATP is related to blood pressure control and circulatory systems via receptors (Circulation Research, Vol. 58(3), pp. 319-330 (1986)) and that ATP and purinoceptors are closely related (Am. Phys. Soc., pp.
15 C577-C606 (1993)).

When the compound or the salt thereof obtained by the screening method or by the screening kit is used as the above-mentioned pharmaceutical composition, a conventional means may be applied therefor. The compound or the salt thereof may be orally, parenterally, by inhalation spray, rectally, or topically administered as pharmaceutical compositions or formulations (e.g. powders, granules, tablets, pills, capsules, injections, syrups, emulsions, elixirs, suspensions, solutions, etc.). For example, it may be used
20 by an oral route as tablets (sugar-coated if necessary), capsules, elixirs, microcapsules, etc. or by a parenteral route as injections such as an aseptic solution or a suspension in water or in other pharmaceutically acceptable liquid. The pharmaceutical compositions or formulations may
25 comprise at least one such compound alone or in admixture with pharmaceutically acceptable carriers, adjuvants, vehicles, excipients and/or diluents. The pharmaceutical compositions may be formulated in accordance with conventional methods. For example, said compound or the salt thereof is mixed in a
30 unit dose form which is required for preparing a generally approved pharmaceutical preparations together with a
35

physiologically acceptable carriers, flavoring and/or perfuming agents (fragrances), fillers, vehicles, antiseptics, stabilizers, binders, etc. whereupon the preparation can be manufactured. An amount of the effective component in those 5 preparations is to be in such an extent that the suitable dose within an indicated range is achieved.

Examples of the additives which can be admixed in the tablets, capsules, etc. are binders such as gelatin, corn starch, tragacanth and gum arabicum; fillers such as 10 crystalline cellulose; swelling agents such as corn starch, gelatin and alginic acid; lubricants such as magnesium stearate; sweetening agents such as sucrose, lactose and saccharine; preservatives such as parabens and sorbic acid; antioxidants such as ascorbic acid, α -tocopherol and cysteine; 15 fragrances such as peppermint, akamono oil and cherry; disintegrants; buffering agents; etc. Other additives may include mannitol, maltitol, dextran, agar, chitin, chitosan, pectin, collagen, casein, albumin, synthetic or semi-synthetic polymers, glyceride, lactide, etc. When the unit form of the 20 preparation is a capsule, a liquid carrier such as fat/oil may be further added besides the above-mentioned types of materials. The aseptic composition for injection may be formulated by a conventional technique or practice for the preparations such as that the active substance in a vehicle 25 such as water for injection is dissolved or suspended in a naturally occurring plant oil such as sesame oil and palm oil.

Examples of an aqueous liquid for the injection are a physiological saline solution and isotonic solutions containing glucose and other auxiliary agents (e.g. D-sorbitol, 30 D-mannitol, sodium chloride, etc.) wherein a suitable auxiliary solubilizers such as alcohol (e.g. ethanol, etc.), polyalcohol (e.g. propylene glycol, polyethylene glycol, etc.), nonionic surface-active agent (e.g. Polysorbate 80TM, HCO-50, etc.), etc. may be jointly used. In the case of the oily liquid, 35 sesame oil, soybean oil, etc. may be exemplified wherein benzyl benzoate, benzyl alcohol, etc. may be jointly used as auxiliary solubilizers.

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In addition, buffers (e.g. phosphate buffer, sodium acetate buffer, etc.), analgesic agents (e.g. benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g. human serum albumin, polyethylene glycol, etc.), stabilizers (e.g. benzyl alcohol, phenol, etc.), antioxidants, etc. may be compounded therewith too. The prepared injection solution is filled in suitable ampoules. The formulation prepared as such is safe and less toxic and, therefore, it can be administered to warm-blooded mammals such as rats, rabbits, sheep, swines, cattle, cats, dogs, monkeys, human being, etc.

Dose levels of said compound or the salt thereof may vary depending upon the symptom. Specific dose levels for any particular patient will be employed depending upon a variety of factors including the activity of specific compounds employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy. In the case of oral administration, it is usually about 0.1-100 mg, preferably about 1.0-50 mg or, more preferably, about 1.0-20 mg per day for adults (as 60 kg). When it is administered parenterally, its dose at a time may vary depending upon the object to be administered, organs to be administered, symptoms, administering methods, etc. The term "parenteral" as used herein includes subcutaneous injections, intravenous, intramuscular, intraperitoneal injections, or infusion techniques. In the case of injections, it is usually convenient to give by an intravenous route in an amount of about 0.01-30 mg, preferably about 0.1-20 mg or, more preferably, about 0.1-10 mg per day to adults (as 60 kg). In the case of other animals, the dose calculated for 60 kg may be administered as well.

(6) Manufacture of Antibody or Antiserum against the G Protein Coupled Receptor Protein of the Present Invention, Its Peptide Fragment or Its Salt.

Antibodies (e.g. polyclonal antibody and monoclonal antibody) and antisera against the G protein coupled receptor

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protein or salt thereof of the present invention or against the peptide fragment of the G protein coupled receptor protein or salt thereof of the present invention may be manufactured by antibody or antiserum-manufacturing methods per se known to
5 those of skill in the art or methods similar thereto, using the G protein coupled receptor protein or its salt of the present invention or the peptide fragment of the G protein coupled receptor protein or its salt of the present invention.
For example, monoclonal antibodies can be manufactured by the
10 method as given below.

[Preparation of Monoclonal Antibody]

(a) Preparation of Monoclonal Antibody-Producing Cells.

The G protein coupled receptor protein of the present invention or its salt or the peptide fragment of the G protein coupled receptor protein of the present invention or its salt (hereinafter, may be abbreviated as the "G protein coupled receptor protein") is administered to warm-blooded animals either solely or together with carriers or diluents to the site where the production of antibody is possible by the administration. In order to potentiate the antibody productivity upon the administration, complete Freund's adjuvants or incomplete Freund's adjuvants may be administered. The administration is usually carried out once every two to six weeks and two to ten times in total.
25 Examples of the applicable warm-blooded animals are monkeys, rabbits, dogs, guinea pigs, mice, rats, sheep, goats and chickens and the use of mice and rats is preferred.

In the preparation of the cells which produce monoclonal antibodies, an animal wherein the antibody titer is noted is selected from warm-blooded animals (e.g. mice) immunized with antigens, then spleen or lymph node is collected after two to five days from the final immunization and antibody-producing cells contained therein are fused with myeloma cells to give monoclonal antibody-producing hybridomas. Measurement of the antibody titer in antisera may, for example, be carried out by reacting a labeled

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G protein coupled receptor protein (which will be mentioned later) with the antiserum followed by measuring the binding activity of the labeling agent with the antibody.

The operation for fusing may be carried out, for example, by
5 a method of Kohler and Milstein (Nature, 256, 495, 1975). Examples of the fusion accelerator are polyethylene glycol (PEG), Sendai virus, etc. and the use of PEG is preferred.

Examples of the myeloma cells are NS-1, P3U1, SP2/0, AP-1, etc. and the use of P3U1 is preferred. The preferred
10 fusion ratio of the numbers of antibody-producing cells used (spleen cells) to the numbers of myeloma cells is within a range of about 1:1 to 20:1. When PEG (preferably, PEG 1000 to PEG 6000) is added in a concentration of about 10-80% followed by incubating at 20-40°C (preferably, at 30-37°C)
15 for one to ten minutes, an efficient cell fusion can be carried out.

Various methods may be applied for screening a hybridoma which produces anti-G protein coupled receptor antibody. For example, a supernatant liquid of hybridoma culture is added to a solid phase (e.g. microplate) to which the G protein coupled receptor protein antigen is adsorbed either directly or with a carrier, then anti-immunoglobulin antibody (anti-mouse immunoglobulin antibody is used when the cells used for the cell fusion are those of mouse) which is labeled with a radioactive substance, an enzyme or the like, or protein A is added thereto and then anti-G protein coupled receptor monoclonal antibodies bound on the solid phase are detected; or a supernatant liquid of the hybridoma culture is added to the solid phase to which
20 anti-immunoglobulin or protein A is adsorbed, then the G protein coupled receptor labeled with a radioactive substance or an enzyme is added and anti-G protein coupled receptor monoclonal antibodies bonded with the solid phase is detected.
25

Selection and cloning of the anti-G protein coupled receptor monoclonal antibody-producing hybridoma may be carried out by methods per se known to those of skill in the art or methods similar thereto. Usually, it is carried out in a

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medium for animal cells, containing HAT (hypoxanthine, aminopterin and thymidine). With respect to a medium for the selection, for the cloning and for the growth, any medium may be used so far as hybridoma is able to grow therein. Examples 5 of the medium are an RPMI 1640 medium (Dainippon Pharmaceutical Co., Ltd., Japan) containing 1-20% (preferably 10-20%) of fetal calf serum (FCS), a GIT medium (Wako Pure Chemical, Japan) containing 1-20% of fetal calf serum and a serum-free medium for hybridoma culturing (SFM-101; Nissui Seiyaku, Japan).

10 The culturing temperature is usually 20-40°C and, preferably, about 37°C. The culturing time is usually from five days to three weeks and, preferably, one to two weeks. The culturing is usually carried out in 5% carbon dioxide gas. The antibody titer of the supernatant liquid of the hybridoma culture may be

15 measured by the same manner as in the above-mentioned measurement of the antibody titer of the anti-G protein coupled receptor in the antiserum.

The cloning can be usually carried out by methods known per se such as techniques in semi-solid agar and 20 limiting dilution. The cloned hybridoma is preferably cultured in modern serum-free culture media to obtain optimal amounts of antibody in supernatants. The target monoclonal antibody is also preferably obtained from ascitic fluid derived from a mouse, etc. injected intraperitoneally with 25 live hybridoma cells.

(b) Purification of the Monoclonal Antibody.

Like in the separation/purification of conventional polyclonal antibodies, the separation/purification of the anti-G protein coupled receptor monoclonal antibody may be 30 carried out by methods for separating/purifying immunoglobulin (such as salting-out, precipitation with an alcohol, isoelectric precipitation, electrophoresis, adsorption/deadsorption using ion exchangers such as DEAE, ultracentrifugation, gel filtration, specific purifying 35 methods in which only an antibody is collected by treatment with an active adsorbent (such as an antigen-binding solid

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phase, protein A or protein G) and the bond is dissociated whereupon the antibody is obtained.

The G protein coupled receptor antibody of the present invention which is manufactured by the aforementioned method (a) or (b) is capable of specifically recognizing G protein coupled receptors and, accordingly, it can be used for a quantitative determination of the G protein coupled receptor in test liquid samples and particularly for a quantitative determination by sandwich immunoassays.

Thus, the present invention provides, for example, the following methods:

- (i) a quantitative determination of a G protein coupled receptor in a test liquid sample, which comprises
 - (a) competitively reacting the test liquid sample and a labeled G protein coupled receptor with an antibody which reacts with the G protein coupled receptor of the present invention, and
 - (b) measuring the ratio of the labeled G protein coupled receptor binding with said antibody; and
- (ii) a quantitative determination of a G protein coupled receptor in a test liquid sample, which comprises
 - (a) reacting the test liquid sample with an antibody immobilized on an insoluble carrier and a labeled antibody simultaneously or continuously, and
 - (b) measuring the activity of the labeling agent on the insoluble carrier

wherein one antibody is capable of recognizing the N-terminal region of the G protein coupled receptor while another antibody is capable of recognizing the C-terminal region of the G protein coupled receptor.

When the monoclonal antibody of the present invention recognizing a G protein coupled receptor (hereinafter, may be referred to as "anti-G protein coupled receptor antibody") is used, G protein coupled receptors can be measured and, moreover, can be detected by means of a tissue staining, etc. as well. For such an object, antibody molecules per se may be used or F(ab')₂, Fab' or Fab fractions of the antibody

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molecule may be used too. There is no particular limitation for the measuring method using the antibody of the present invention and any measuring method may be used so far as it relates to a method in which the amount of antibody, antigen or 5 antibody-antigen complex, depending on or corresponding to the amount of antigen (e.g. the amount of G protein coupled receptor, etc.) in the liquid sample to be measured, is detected by a chemical or a physical means and then calculated using a standard curve prepared by a standard solution 10 containing the known amount of antigen. For example, nephrometry, competitive method, immunometric method and sandwich method are suitably used and, in terms of sensitivity and specificity, the sandwich method which will be described herein later is particularly preferred.

15 Examples of the labeling agent used in the measuring method using the labeling substance are radioisotopes, enzymes, fluorescent substances, luminescent substances, colloids, magnetic substances, etc. Examples of the radioisotope are [¹²⁵I], [¹³¹I], [³H] and [¹⁴C]; 20 preferred examples of the enzyme are those which are stable and with big specific activity, such as β -galactosidase, β -glucosidase, alkali phosphatase, peroxidase and malate dehydrogenase; examples of the fluorescent substance are fluorescamine, fluorescein 25 isothiocyanate, etc.; and examples of the luminescent substance are luminol, luminol derivatives, luciferin, lucigenin, etc. Further, a biotin-avidin system may also be used for binding an antibody or antigen with a labeling agent.

In an insolubilization (immobilization) of antigens 30 or antibodies, a physical adsorption may be used or a chemical binding which is usually used for insolubilization or immobilization of proteins or enzymes may be used as well. Examples of the carrier are insoluble polysaccharides such as agarose, dextran and cellulose; synthetic resins such as 35 polystyrene, polyacrylamide and silicone; glass; etc.

In a sandwich (or two-site) method, the test liquid is made to react with an insolubilized anti-G protein coupled

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receptor antibody (the first reaction), then it is made to react with a labeled anti-G protein coupled receptor antibody (the second reaction) and the activity of the labeling agent on the insoluble carrier is measured whereupon the amount of 5 the G protein coupled receptor in the test liquid can be determined. The first reaction and the second reaction may be conducted reversely or simultaneously or they may be conducted with an interval. The type of the labeling agent and the method of insolubilization (immobilization) may be the same as 10 those mentioned already herein. In the immunoassay by means of a sandwich method, it is not always necessary that the antibody used for the labeled antibody and the antibody for the solid phase is one type or one species but, with an object 15 of improving the measuring sensitivity, etc., a mixture of two or more antibodies may be used too.

In the method of measuring G protein coupled receptors by the sandwich method of the present invention, the preferred anti-G protein coupled receptor antibodies used for the first and the second reactions are antibodies wherein 20 their sites binding to the G protein coupled receptors are different each other. Thus, the antibodies used in the first and the second reactions are those wherein, when the antibody used in the second reaction recognizes the C-terminal region of the G protein coupled receptor, then the antibody 25 recognizing the site other than C-terminal regions, e.g. recognizing the N-terminal region, is preferably used in the first reaction.

The anti-G protein coupled receptor antibody of the present invention may be used in a measuring system other than 30 the sandwich method such as a competitive method, an immunometric method and a nephrometry. In a competitive method, an antigen in the test solution and a labeled antigen are made to react with an antibody in a competitive manner, then an unreacted labeled antigen (F) and a labeled 35 antigen binding with an antibody (B) are separated (i.e. B/F separation) and the labeled amount of any of B and F is measured whereupon the amount of the antigen in the test

solution is determined. With respect to a method for such a reaction, there are a liquid phase method in which a soluble antibody is used as the antibody and the B/F separation is conducted by polyethylene glycol, a second antibody to the 5 above-mentioned antibody, etc.; and a solid phase method in which an immobilized antibody is used as the first antibody or a soluble antibody is used as the first antibody while an immobilized antibody is used as the second antibody.

In an immunometric method, an antigen in the test 10 solution and an immobilized antigen are subjected to a competitive reaction with a certain amount of a labeled antibody followed by separating into solid and liquid phases; or the antigen in the test solution and an excess amount of labeled antibody are made to react, then a immobilized antigen 15 is added to bind an unreacted labeled antibody with the solid phase and separated into solid and liquid phases. After that, the labeled amount of any of the phases is measured to determine the antigen amount in the test solution.

In a nephrometry, the amount of insoluble sediment 20 which is produced as a result of the antigen-antibody reaction in a gel or in a solution is measured. Even when the antigen amount in the test solution is small and only a small amount of the sediment is obtained, a laser nephrometry wherein scattering of laser is utilized can be suitably used.

In applying each of those immunological measuring 25 methods (immunoassays) to the measuring method of the present invention, it is not necessary to set up any special condition, operation, etc. therefor. A measuring system (assay system) for G protein coupled receptor may be constructed taking the technical consideration of the persons skilled in the art into 30 consideration in the conventional conditions and operations for each of the methods. With details of those conventional technical means, a variety of reviews, reference books, etc. may be referred to. They are, for example, Hiroshi Irie (ed): "Radioimmunoassay" (Kodansha, Japan, 1974); Hiroshi Irie (ed): "Radioimmunoassay; Second Series" (Kodansha, Japan, 1979); 35 Eiji Ishikwa et al. (ed): "Enzyme Immunoassay" (Igaku Shoin,

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Japan, 1978); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay" (Second Edition) (Igaku Shoin, Japan, 1982); Eiji Ishikawa et al. (ed): "Enzyme Immunoassay" (Third Edition) (Igaku Shoin, Japan, 1987); "Methods in Enzymology" Vol. 70 (Immunochemical Techniques (Part A)); ibid. Vol. 73 (Immunochemical Techniques (Part B)); ibid. Vol. 74 (Immunochemical Techniques (Part C)); ibid. Vol. 84 (Immunochemical Techniques (Part D: Selected Immunoassays)); ibid. Vol. 92 (Immunochemical Techniques (Part E: Monoclonal Antibodies and General Immunoassay Methods)); ibid. Vol. 121 (Immunochemical Techniques (Part I: Hybridoma Technology and Monoclonal Antibodies)) (Academic Press); etc.

(7) Preparation of Animals Having the G Protein Coupled Receptor Protein-Encoding DNA of the Present Invention.

It is possible to prepare transgenic animals expressing G protein coupled receptors using G protein coupled receptor protein-encoding DNA. Examples of the animals are warm-blooded mammals such as rats, rabbit, sheep, swines, cattle, cats, dogs and monkeys.

In transferring the G protein coupled receptor protein-encoding DNA to the aimed animal, it is generally advantageous that said DAN is used by ligating with a site at the downstream of a promoter which is capable of expressing in animal cells. For example, when G protein coupled receptor protein DNA is to be transferred to a rabbit, a gene construct ligated with a site at the downstream of various promoters which are capable of expressing the G protein coupled receptor protein DNA derived from an animal compatible to the animal in animal host cells is subjected to a microinjection to the fertilized ovum (oosperm) of the aimed animal (e.g. fertilized ovum (embryo) of rabbit) whereupon the transgenic animal which produces the G protein coupled receptor protein in a high amount can be prepared.

Examples of the promoters used are promoters derived from virus and ubiquitous expression promoters such as metallothionein promoters may be used but, preferably,

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enolase gene promoters and NGF gene promoters capable of specifically expressing in brain are used.

Transfer of the G protein coupled receptor protein DNA at a fertilized ovum cell stage is secured in order that 5 the DNA can be present in all of embryonal cells and body somatic cells of an aimed animal. The fact that the G protein coupled receptor protein is present in the fertilized ovum cells of the produced transgenic animal after the DNA transfer means that all progeny of the produced transgenic animal 10 have the G protein coupled receptor protein in all of their embryonal cells and somatic cells. Descendants (offsprings) of the animal of this type which inherited the gene have the G protein coupled receptor protein in all of their embryonal cells and somatic cells.

15 The transgenic animal to which the G protein coupled receptor protein DNA is transferred can be subjected to a mating and a breeding for generations under a common breeding circumstance as the animal holding said DNA after confirming that the gene can be stably retained. Moreover, male and 20 female animals having the desired DNA are mated to give a homozygote having the transduced gene in both homologous chromosomes and then those male and female animals are mated whereby it is possible to breed for generations so that all descendants have said DNA.

25 The animal to which the G protein coupled receptor protein DNA is transferred highly expresses the G protein coupled receptor protein and, accordingly, it is useful as the animal for screening for an agonist or an antagonist to said G protein coupled receptor protein.

30 The DNA-transferred animal can be used as a cell source for a tissue culture. For example, DNA or RNA in the tissue of the DNA-transferred mouse is directly analyzed or protein tissues expressed by gene are analyzed whereupon the G protein coupled receptor protein can be analyzed. 35 Cells of the G protein coupled receptor protein-containing tissue are cultured by standard tissue culture techniques whereupon it is possible to study the function of the cells

which are usually difficult to culture (e.g. those derived from brain and peripheral tissues) using the resulting culture. By using said cells, it is also possible to select the pharmaceuticals which can potentiate, for example, the functions of various tissues. Moreover, if a cell strain with a high expression is available, it is possible to separate and purify G protein coupled receptor proteins therefrom.

As such, the amount of G protein coupled receptor proteins can now be determined with a high precision using the anti-G protein coupled receptor antibody of the present invention.

(8) Antisense Oligonucleotides Capable of Inhibiting Replication of G Protein Coupled Receptor Protein Gene

In another aspect of the present invention, antisense oligonucleotides (nucleic acids) capable of inhibiting the replication or expression of G protein coupled receptor protein gene may be designed and synthesized based on information on the nucleotide sequences of cloned and determined G protein coupled receptor protein-encoding DNAs. Such an antisense oligonucleotide (nucleic acid) is capable of hybridizing with RNA of G protein coupled receptor protein genes to inhibit the synthesis or function of said RNA or of modulating the expression of a G protein coupled receptor protein gene via interaction with G protein coupled receptor protein-related RNA. Oligonucleotides complementary to, and specifically hybridizable with, selected sequences of G protein coupled receptor protein-related RNA are useful in controlling or modulating the expression of a G protein coupled receptor protein gene in vitro and in vivo, and in treating or diagnosing disease states of suspected animals. The term "corresponding" means homologous to or complementary to a particular sequence of the nucleotide sequence or nucleic acid including the gene. As between nucleotides (nucleic acids) and peptides (proteins), "corresponding" usually refers to amino acids of a peptide (protein) in an order derived from the sequence of a nucleotides (nucleic acids) or its complement.

The G protein coupled receptor protein gene 5' end hairpin loop, 5' end 6-base-pair repeats, 5' end untranslated region, polypeptide translation initiation codon, protein coding region, ORF translation initiation codon, 3' untranslated region, 3' end palindrome region, and 3' end hairpin loop may be selected as preferred targets though any region may be a target among G protein coupled receptor protein genes.

5 The relationship between the target and oligonucleotides complementary to at least a portion of the target, specifically hybridizable with the target, is denoted as "antisense".

10 The antisense oligonucleotides may be polydeoxynucleotides containing 2-deoxy-D-ribose, polyribonucleotides containing D-ribose, any other type of polynucleotide which is an N-glycoside of a purine or pyrimidine base, or other polymers

15 containing nonnucleotide backbones (e.g., protein nucleic acids and synthetic sequence-specific nucleic acid polymers commercially available) or nonstandard linkages, providing that the polymers contain nucleotides in a configuration which allows for base pairing and base stacking such as is found in

20 DNA and RNA. They may include double- and single-stranded DNA, as well as double- and single-stranded RNA and DNA:RNA hybrids, and also include, as well as unmodified forms of the polynucleotide or oligonucleotide, known types of modifications, for example, labels which are known to those

25 skilled in the art, "caps", methylation, substitution of one or more of the naturally occurring nucleotides with analogue, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.) and with

30 charged linkages or sulfur-containing linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (including nucleases, nuclease inhibitors, toxins, antibodies, signal peptides, poly-L-lysine, etc.) and saccharides (e.g., monosaccharides, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.),

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those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.). The terms "nucleoside", "nucleotide" and "nucleic acid" will include those moieties which contain not only the known purine and 5 pyrimidine bases, but also other heterocyclic bases which have been modified. Such modifications include methylated purines and pyrimidines, acylated purines and pyrimidines, or other heterocycles. Modified nucleosides or nucleotides will also include modifications on the sugar moiety, e.g., wherein one or 10 more of the hydroxyl groups are replaced with halogen, aliphatic groups, or are functionalized as ethers, amines, or the like.

The antisense nucleic acid of the present invention is RNA, DNA or a modified nucleic acid. Examples of modified 15 nucleic acid are, but not limited to, degradation-resistant sulfurized and thiophosphate derivatives of nucleic acids, and poly- or oligonucleoside amides. Preferred design modifications of the antisense nucleic acids of the present invention are modifications that are designed to:

- 20 (1) increase the intracellular stability of the nucleic acid;
- (2) increase the cellular permeability of the nucleic acid;
- (3) increase the affinity of the nucleic acid for the target sense strand; or

- (4) decrease the toxicity (if any) of the nucleic acid.

25 Many such modifications are known to those skilled in the art, as described in J. Kawakami et al., Pharm Tech Japan, Vol. 8, pp.247, 1992; Vol. 8, pp.395, 1992; S. T. Crooke et al. ed., Antisense Research and Applications, CRC Press, 1993; etc. The nucleic acids may contain altered or modified sugars, bases 30 or linkages, be delivered in specialized systems such as liposomes, microspheres or by gene therapy, or may have attached moieties. Such attached moieties include polycationic moieties such as polylysine that act as charge neutralizers of the phosphate backbone, or hydrophobic moieties such as lipids 35 (e.g., phospholipids, cholesterol, etc.) that enhance interaction with cell membranes or increase uptake of the nucleic acid. Preferred lipids that may attached are

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cholesterols or derivatives thereof (e.g., cholesteryl chloroformate, cholic acid, etc.). The moieties may be attached at the 3' or 5' ends of the nucleic acids, and also may be attached through a base, sugar, or internucleoside
5 linkage. Other moieties may be capping groups specifically placed at the 3' or 5' ends of the nucleic acids to prevent degradation by nuclease such as exonuclease, RNase, etc. Such capping groups include, but are not limited to, hydroxyl protecting groups known to those skilled in the art, including
10 glycols such as polyethylene glycols, tetraethylene glycol and the like.

The inhibitory activity of antisense nucleic acids can be examined using the transformant (or transfectant) of the present invention, the in vitro and in vivo gene
15 expression system of the present invention, or the in vitro and in vivo translation system of G protein coupled receptor proteins. The nucleic acid can be placed in the cell through any number of ways known per se.

In the specification and drawings of the present
20 application, the abbreviations used for bases (nucleotides), amino acids and so forth are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature or those conventionally used in the art. Examples thereof are given below.
Amino acids for which optical isomerism is possible are, unless
25 otherwise specified, in the L form.

DNA : Deoxyribonucleic acid
cDNA: Complementary deoxyribonucleic acid
A : Adenine
T : Thymine
30 G : Guanine
C : Cytosine
RNA : Ribonucleic acid
mRNA : Messenger ribonucleic acid
dATP: Deoxyadenosine triphosphate
35 dTTP: Deoxythymidine triphosphate

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dGTP: Deoxyguanosine triphosphate
dCTP: Deoxycytidine triphosphate
ATP : Adenosine triphosphate
EDTA: Ethylenediamine tetraacetic acid

5 SDS : Sodium dodecyl sulfate
EIA: Enzyme Immunoassay

G, Gly: Glycine (or Glycyl)
A, Ala: Alanine (or Alanyl)
V, Val: Valine (or Valyl)

10 L, Leu: Leucine (or Leucyl)
I, Ile: Isoleucine (or Isoleucyl)
S, Ser: Serine (or Seryl)
T, Thr: Threonine (or Threonyl)
C, Cys: Cysteine (or Cysteinyl)

15 M, Met: Methionine (or Methionyl)
E, Glu: Glutamic acid (or Glutamyl)
D, Asp: Aspartic acid (or Aspartyly)
K, Lys: Lysine (or Lysyl)
R, Arg: Arginine (or Arginyl)

20 H, His: Histidine (or Histidyl)
F, Phe: Phenylalanine (or Phenylalanyl)
Y, Tyr: Tyrosine (or Tyrosyl)
W, Trp: Tryptophan (or Tryptophanyl)
P, Pro: Proline (or Prolyl)

25 N, Asn: Asparagine (or Asparaginyl)
Q, Gln: Glutamine (or Glutaminy)
NVal: Norvaline (or Norvalyl)
pGlu: Pyroglutamic acid (or Pyroglutamyl)
Blc: γ -Butyrolacton- γ -carbonyl

30 Kpc: 2-Ketopiperidinyl-6-carbonyl
Otc: 3-Oxoperhydro-1,4-thiazin-5-carbonyl
Me: Methyl
Et: Ethyl
Bu: Butyl

35 Ph: Phenyl
TC: Thiazolidinyl-4(R)-carboxamide

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The transformant *Escherichia coli*, designated INV α F'/p19P2, which is obtained in the Example 3 mentioned herein below, is on deposit under the terms of the Budapest Treaty from August 9, 1994, with the National Institute of Bioscience and Human-Technology (NIBH), Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan and has been assigned the Accession Number 5 FERM BP-4776. It is also on deposit from August 22, 1994 with the Institute for Fermentation, Osaka, Japan (IFO) and has been assigned the Accession Number IFO 10 15739.

The transformant *Escherichia coli*, designated INV α F'/pG3-2, which is obtained in the Example 4 mentioned herein below, is on deposit under the terms of the Budapest Treaty from August 9, 1994, with NIBH and has been assigned 15 the Accession Number FERM BP-4775. It is also on deposit from August 22, 1994 with IFO and has been assigned the Accession Number IFO 15740.

The transformant *Escherichia coli*, designated INV α F'/p63A2, which is obtained in the Example 5 mentioned 20 herein below, is on deposit under the terms of the Budapest Treaty from August 9, 1994, with NIBH and has been assigned the Accession Number FERM BP-4777. It is also on deposit from August 22, 1994 with IFO and has been assigned the Accession Number IFO 15738.

25 The transformant *Escherichia coli*, designated JM109/phGR3, which is obtained in the Example 6 mentioned herein below, is on deposit under the terms of the Budapest Treaty from September 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4807. It is also on deposit from 30 September 22, 1994 with IFO and has been assigned the Accession Number IFO 15748.

The transformant *Escherichia coli*, designated JM109/p3H2-17, which is obtained in the Example 7 mentioned 35 herein below, is on deposit under the terms of the Budapest Treaty from September 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4806. It is also on deposit from September 22, 1994 with IFO and has been assigned the Accession

Number IFO 15747.

The transformant *Escherichia coli*, designated JM109/p3H2-34, which is obtained in the Example 8 mentioned herein below, is on deposit under the terms of the Budapest Treaty from October 12, 1994, with NIBH and has been assigned the Accession Number FERM BP-4828. It is also on deposit from October 12, 1994 with IFO and has been assigned the Accession Number IFO 15749.

The transformant *Escherichia coli*, designated JM109/pMD4, which is obtained in the Example 9 mentioned herein below, is on deposit under the terms of the Budapest Treaty from November 11, 1994, with NIBH and has been assigned the Accession Number FERM BP-4888. It is also on deposit from November 17, 1994 with IFO and has been assigned the Accession Number IFO 15765.

The transformant *Escherichia coli*, designated JM109/pMGR20, which is obtained in the Example 10 mentioned herein below, is on deposit under the terms of the Budapest Treaty from December 15, 1994, with NIBH and has been assigned the Accession Number FERM BP-4937. It is also on deposit from December 14, 1994 with IFO and has been assigned the Accession Number IFO 15773.

The transformant *Escherichia coli*, designated JM109/pMJ10, which is obtained in the Example 12 mentioned herein below, is on deposit under the terms of the Budapest Treaty from December 15, 1994, with NIBH and has been assigned the Accession Number FERM BP-4936. It is also on deposit from December 16, 1994 with IFO and has been assigned the Accession Number IFO 15784.

The transformant *Escherichia coli*, designated JM109/pMH28, which is obtained in the Example 14 mentioned herein below, is on deposit under the terms of the Budapest Treaty from January 13, 1995, with NIBH and has been assigned the Accession Number FERM BP-4970. It is also on deposit from January 20, 1995 with IFO and has been assigned the Accession Number IFO 15791.

The transformant *Escherichia coli*, designated

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JM109/pMN7, which is obtained in the Example 16 mentioned herein below, is on deposit under the terms of the Budapest Treaty from February 22, 1995, with NIBH and has been assigned the Accession Number FERM BP-5011. It is also on deposit from 5 February 27, 1995 with IFO and has been assigned the Accession Number IFO 15803.

The transformant *Escherichia coli*, designated JM109/p5S38, which is obtained in the Example 17 mentioned herein below, is on deposit under the terms of the Budapest 10 Treaty from October 27, 1994, with NIBH and has been assigned the Accession Number FERM BP-4856. It is also on deposit from October 25, 1994 with IFO and has been assigned the Accession Number IFO 15754.

The transformant *Escherichia coli*, designated 15 JM109/pMAH2-17, which is obtained in the Example 19 mentioned herein below, is on deposit under the terms of the Budapest Treaty from April 7, 1995, with NIBH and has been assigned the Accession Number FERM BP-5073. It is also on deposit from March 31, 1995 with IFO and has been assigned the Accession 20 Number IFO 15813.

The transformant *Escherichia coli*, designated JM109/pMN128, which is obtained in the Example 20 mentioned herein below, is on deposit under the terms of the Budapest Treaty from March 17, 1995, with NIBH and has been assigned the 25 Accession Number FERM BP-5039. It is also on deposit from March 22, 1995 with IFO and has been assigned the Accession Number IFO 15810.

The transformant *Escherichia coli*, designated JM109/phAH2-17, which is obtained in the Example 21 mentioned 30 herein below, is on deposit under the terms of the Budapest Treaty from July 20, 1995, with NIBH and has been assigned the Accession Number FERM BP-5168. It is also on deposit from July 14, 1995 with IFO and has been assigned the Accession Number IFO 15856.

35 Each SEQ ID NO set forth in the SEQUENCE LISTING of the specification refers to the following sequence:

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[SEQ ID NO: 24] is a partial amino acid sequence of the human pituitary gland-derived G protein coupled receptor protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2,

5 [SEQ ID NO: 25] is a partial amino acid sequence of the human pituitary gland-derived G protein coupled receptor protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2,

[SEQ ID NO: 26] is an entire amino acid sequence of the human pituitary gland-derived G protein coupled receptor protein encoded by the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in phGR3,

10 [SEQ ID NO: 27] is a partial amino acid sequence of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein encoded by the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA fragment having a nucleotide sequence (SEQ ID NO: 32), derived based upon the nucleotide sequences of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA

15 fragments each included in pG3-2 and pG1-10,

[SEQ ID NO: 28] is a partial amino acid sequence of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein encoded by p5S38,

[SEQ ID NO: 29] is a nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2,

20 [SEQ ID NO: 30] is a nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA fragment included in p19P2,

[SEQ ID NO: 31] is an entire nucleotide sequence of the human pituitary gland-derived G protein coupled receptor protein cDNA included in phGR3,

25 [SEQ ID NO: 32] is a nucleotide sequence of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA, derived based upon the nucleotide sequences of the mouse pancreatic β -cell line, MIN6-derived G protein coupled receptor protein cDNA fragments each

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included in pG3-2 and pG1-10,
[SEQ ID NO: 33] is a nucleotide sequence of the mouse
pancreatic β -cell line, MIN6-derived G protein cDNA included
in p5S38,

5 [SEQ ID NO: 34] is a partial amino acid sequence of the human
amygdaloid nucleus-derived G protein coupled receptor protein
encoded by the cDNA fragment included in p63A2,
[SEQ ID NO: 35] is a partial amino acid sequence of the human
amygdaloid nucleus-derived G protein coupled receptor protein
10 encoded by the cDNA fragment included in p63A2,
[SEQ ID NO: 36] is a nucleotide sequence of the human
amygdaloid nucleus-derived G protein coupled receptor protein
cDNA fragment included in p63A2,
[SEQ ID NO: 37] is a nucleotide sequence of the human
15 amygdaloid nucleus-derived G protein coupled receptor protein
cDNA fragment included in p63A2,
[SEQ ID NO: 38] is a partial amino acid sequence encoded
by the mouse pancreatic β -cell line, MIN6-derived G protein
coupled receptor protein cDNA included in p3H2-17,
20 [SEQ ID NO: 39] is a full-length amino acid sequence encoded
by the open reading frame of the mouse pancreatic β -cell line,
MIN6-derived G protein coupled receptor protein cDNA included
in pMAH2-17,
[SEQ ID NO: 40] is a nucleotide sequence of the mouse
25 pancreatic β -cell line, MIN6-derived G protein coupled
receptor protein cDNA included in p3H2-17,
[SEQ ID NO: 41] is a nucleotide sequence of the mouse
pancreatic β -cell line, MIN6-derived G protein coupled
receptor protein cDNA included in pMAH2-17,
30 [SEQ ID NO: 42] is a partial amino acid sequence encoded
by the mouse pancreatic β -cell line, MIN6-derived G protein
coupled receptor protein cDNA included in p3H2-34,
[SEQ ID NO: 43] is a nucleotide sequence of the mouse
pancreatic β -cell line, MIN6-derived G protein coupled
35 receptor protein cDNA fragment included in p3H2-34,
[SEQ ID NO: 44] is a partial amino acid sequence encoded
by the rabbit gastropyloric part smooth muscle-derived G

- 1 8 8 -

protein coupled receptor protein cDNA included in pMD4,
[SEQ ID NO: 45] is a nucleotide sequence of the rabbit
gastropylolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMD4,

5 [SEQ ID NO: 46] is an entire amino acid sequence
encoded by the mouse pancreatic β -cell line, MIN6-derived G
protein coupled receptor protein cDNA included in pMGR20,
[SEQ ID NO: 47] is a nucleotide sequence of the mouse
pancreatic β -cell line, MIN6-derived G protein coupled
10 receptor protein cDNA included in pMGR20,

[SEQ ID NO: 48] is a partial amino acid sequence encoded
by the rabbit gastropylolic part smooth muscle-derived G
protein coupled receptor protein cDNA included in pMJ10,
[SEQ ID NO: 49] is a nucleotide sequence of the rabbit
15 gastropylolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMJ10,
[SEQ ID NO: 50] is a partial amino acid sequence encoded
by the rabbit gastropylolic part smooth muscle-derived G
protein coupled receptor protein cDNA included in pMH28,
20 [SEQ ID NO: 51] is a nucleotide sequence of the rabbit
gastropylolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMH28,
[SEQ ID NO: 52] is a partial amino acid sequence encoded
by the rabbit gastropylolic part smooth muscle-derived G
25 protein coupled receptor protein cDNA included in pMN7,
[SEQ ID NO: 53] is a nucleotide sequence of the rabbit
gastropylolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMN7,
[SEQ ID NO: 54] is a partial amino acid sequence encoded
30 by the rabbit gastropylolic part smooth muscle-derived G
protein coupled receptor protein cDNA included in pMN128,
[SEQ ID NO: 55] is a nucleotide sequence of the rabbit
gastropylolic part smooth muscle-derived G protein coupled
receptor protein cDNA fragment included in pMN128,
35 [SEQ ID NO: 56] is a full-length amino acid sequence of the
human-derived G protein coupled receptor protein encoded
by the human-derived G protein coupled receptor protein cDNA

included in phAH2-17, and

[SEQ ID NO: 57] is a nucleotide sequence of the human-derived G protein coupled receptor protein cDNA included in phAH2-17.

EXAMPLES

5 Described below are working examples of the present invention which are provided only for illustrative purposes, and not to limit the scope of the present invention. In light of the present disclosure, numerous embodiments within the scope of the claims will be apparent to those of ordinary skill
10 in the art.

Example 1

Preparation of Synthetic DNA Primer for Amplifying DNA Coding for G Protein Coupled Receptor Protein

15 A comparison of deoxyribonucleotide sequences coding for the known amino acid sequences corresponding to or near the first membrane-spanning domain each of human-derived TRH receptor protein (HTRHR), human-derived RANTES receptor protein (L10918, HUMRANTES), human Burkitt's lymphoma-derived unknown ligand receptor protein (X68149,
20 HSBLR1A), human-derived somatostatin receptor protein (L14856, HUMSOMAT), rat-derived μ -opioid receptor protein (U02083, RNU02083), rat-derived κ -opioid receptor protein (U00442, U00442), human-derived neuromedin B receptor protein (M73482, HUMNMBR), human-derived muscarinic acetylcholine
25 receptor protein (X15266, HSHM4), rat-derived adrenaline α_1 B receptor protein (L08609, RATAADRE01), human-derived somatostatin 3 receptor protein (M96738, HUMSSTR3X), human-derived C₅a receptor protein (HUMC5AAR), human-derived unknown ligand receptor protein (HUMRDC1A), human-derived
30 unknown ligand receptor protein (M84605, HUMOPIODRE) and rat-derived adrenaline α_2 B receptor protein (M91466, RATA2BAR) was made. As a result, highly homologous regions or parts were found (Figure 1).

35 Further, a comparison of deoxynucleotide sequences coding for the known amino acid sequences corresponding to or near the sixth membrane-spanning domain each of mouse-derived

- 1 9 0 -

unknown ligand receptor protein (M80481, MUSGIR), human-derived bombesin receptor protein (L08893, HUMBOMB3S), human-derived adenosine A2 receptor protein (S46950, S46950), mouse-derived unknown ligand receptor protein (D21061, MUSGPCR), mouse-derived TRH receptor protein (S43387, S43387), rat-derived neuromedin K receptor protein (J05189, RATNEURA), rat-derived adenosine A1 receptor protein (M69045, RATA1ARA), human-derived neurokinin A receptor protein (M57414, HUMNEKAR), rat-derived adenosine A3 receptor protein (M94152, RATADENREC), human-derived somatostatin 1 receptor protein (M81829, HUMSRI1A), human-derived neurokinin 3 receptor protein (S86390, S86371S4), rat-derived unknown ligand receptor protein (X61496, RNCGPCR), human-derived somatostatin 4 receptor protein (L07061, HUMSSTR4Z) and rat-derived GnRH receptor protein (M31670, RATGNRHA) was made. As a result, highly homologous regions or parts were found (Figure 2).

The aforementioned abbreviations in the parentheses are identifiers (reference numbers) which are indicated when GenBank/EMBL Data Bank is retrieved by using DNASIS 20 Gene/Protein Sequencing Data Base (CD019, Hitachi Software Engineering, Japan) and are usually called "Accession Numbers" or "Entry Names". HTRHR is, however, the sequence as disclosed in Japanese Unexamined Patent Publication No. 286986/1993 (EPA 638645).

25 Specifically, it was planned to incorporate mixed bases relying upon the base regions that were in agreement with cDNAs coding for a large number of receptor proteins in order to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions. Based upon 30 these sequences, the degenerate synthetic DNA having a nucleotide sequence represented by SEQ ID NO: 1 which is complementary to the homologous nucleotide sequence of Figure 1 and the degenerate synthetic DNA having a nucleotide sequence represented by SEQ ID NO: 2 which is complementary to the 35 homologous nucleotide sequence of Figure 2 were produced. Nucleotide synthesis was carried out by a DNA synthesizer.

- 1 9 1 -

[Synthetic DNAs]

5'-CGTGG (G or C) C (A or C) T (G or C) (G or C) TGGGCAAC
(A, G, C or T) (C or T) CCTG-3'

(SEQ ID NO: 1)

5 5'-GT (A, G, C or T) G (A or T) (A or G) (A or G) GGCA
(A, G, C or T) CCAGCAGA (G or T) GGCAAA-3'

(SEQ ID NO: 2)

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis.

Example 2

15 Isolation of Human Somatostatin Receptor Protein-Encoding DNA,
Human D5 Dopamine Receptor Protein-Encoding DNA, and Rat
Somatostatin Receptor Protein-Encoding DNA

(1) Amplification of DNA by Polymerase Chain Reaction (PCR)
cDNAs (QuickClone, CLONTECH Laboratories, Inc.)
prepared from human brain amygdaloid nucleus, human pituitary
20 gland and rat brain each in an amount of 1 ng as templates, the
synthetic DNA primers prepared in Example 1 each in an amount
of 1 μ M, 2.5 mM dNTPs (deoxyribonucleoside triphosphates), and
2.5 units of Taq DNA polymerase (Takara Shuzo Co., Japan)
were mixed together with a buffer attached to the enzyme kit
25 such that the total amount was 100 μ l. The polymerase chain
reaction was carried out by using a Thermal Cycler manufactured
by Perkin-Elmer Co. One cycle was set to include 96 °C for
30 sec., 45 °C for 1 min. and 60 °C for 3 min.. Totally
this one cycle was repeated 30 times to amplify DNAs.
30 Amplification of DNAs was confirmed by 1.2% agarose
electrophoresis [Figure 17].

- 1 9 2 -

(2) Isolation of Amplified DNA and Analysis of DNA Sequence

By using a TA Cloning Kit (Invitrogen Co.), the DNA amplified by the PCR was inserted into a plasmid vector, pCRTM II. The DNA was transfected into E. coli attached to the 5 kit to form an amplified DNA library. Colonies formed by the transformants were selected under guidance based on the activity of β -galactosidase on X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside)-added LB (Luria-Bertani) plates in order to separate only white colonies in which DNA fragments 10 are inserted. They were cultured in an LB culture medium to which ampicillin was added and plasmid DNAs were prepared with an automatic plasmid extracting machine (Kurabo Co., Japan).

An aliquot of the DNA thus prepared was further digested with EcoRI to confirm DNA fragments that were 15 inserted, and a DNA yield each of clones was compared with a marker. An aliquot of the plasmid DNA thus prepared was treated with RNase, extracted with phenol/chloroform, precipitated in ethanol, and the resulting product was then reacted for sequencing by using a DyeDeoxy terminator cycle 20 sequencing kit (Applied Biosystems Co.).

Sequencing was carried out by using a 370A fluorescent automatic sequencer manufactured by Applied Biosystems Co. The nucleotide sequences obtained were analyzed by using DNASIS (Hitachi Software Engineering, Japan). 25 The nucleotide sequences obtained are shown in Figures 18, 19, 20 and 21. From these Figures and the results of homology retrieval, it was learned that the DNAs obtained were 30 DNAs encoding human somatostatin receptor protein [Figures 18 and 19], human D5 dopamine receptor protein [Figure 20] and rat somatostatin receptor protein [Figure 21] that can be classified each into a group of G protein coupled receptor proteins.

In Figure 18 as described herein, the nucleotide sequence of the DNA is in agreement with the nucleotide 35 sequence encoding somatostatin receptor (HUMSOMAT) and the clone, A58, is a human somatostatin receptor cDNA. The underlined part represents the 5' side synthetic DNA primer

- 1 9 3 -

used for the PCR. Thus, even when parts of the nucleotide sequence are mismatched, amplification is effected to a sufficient degree by the PCR.

It will be understood from Figure 19 that the clone,
5 A58 is in good agreement with the nucleotide sequence coding
for the human somatostatin receptor (HUMSOMAT) even when the
sequencing is carried out from the opposite side. The
underlined part represents the 3' side synthetic DNA primer
used for the PCR. In this figure, the nucleotide sequences are
10 mismatched to some extent even in the portions other than the
primer portion presumably due to base substitution at the time
of PCR or due to partial deviation in the sequencing reaction.
It can be confirmed via sequencing of chains complementary
thereto as required.

15 In Figure 20 as described herein, the nucleotide
sequence of the DNA is in good agreement with a nucleotide
sequence coding for the human D5 dopamine receptor (HUMDRD5A)
except the primer portion (underlined). It was learned that
the clone, 57-A-2, is a human D5 dopamine receptor cDNA.

20 In Figure 21 as described herein, the DNA is in good
agreement with a nucleotide sequence coding for the rat
somatostatin receptor (RNU04738) except the primer portion
(underlined). It was learned that the clone, B54, is a rat
somatostatin receptor cDNA.

25

Example 3

Isolation of Human Pituitary Gland-Derived G Protein Coupled
Receptor Protein-Encoding DNA

(1) Amplification of Receptor cDNA by PCR Using Human
Pituitary Gland-Derived cDNA

30 By using human pituitary gland-derived cDNA
(QuickClone, CLONTECH Laboratories, Inc.) as a template, PCR
amplification using the DNA primers synthesized in Example 1
was carried out. The composition of the reaction solution
consisted of the synthetic DNA primers (SEQ: 5' primer sequence
35 and 3' primer sequence) each in an amount of 1 μ M, 1 ng of the
template cDNA, 0.25 mM dNTPs, 1 μ l of Taq DNA polymerase and

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a buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 95 °C for 1 min., 55 °C for 1 min. and 72 °C for 1 min. was repeated 30 times by using a Thermal Cycler (Perkin-Elmer Co.). Prior to adding Taq DNA polymerase, the remaining reaction solution was mixed and was heated at 95 °C for 5 minutes and at 65 °C for 5 minutes. The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

10 (2) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

The PCR products were separated by using a 0.8% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned into the plasmid vector, pCRTM II (TM represents registered trademark). The recombinant vectors were introduced into E. coli INV α F' competent cells (Invitrogen Co.) to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia coli INV α F'/p19P2.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNA thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNA was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a

- 1 9 5 -

fluorescent automatic sequencer, and the data of the nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan). The underlined portions represent regions corresponding to the synthetic primers [Figures 22 and 23].

Homology retrieval was carried out based upon the determined nucleotide sequences [Figures 22 and 23]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid, p19P2, possessed by the transformant Escherichia coli INV α F'/p19P2. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequences were converted into amino acid sequences [Figures 22 and 23], and homology retrieval was carried out in view of hydrophobicity plotting [Figures 24 and 25] and at the amino acid sequence level to find homology relative to neuropeptide Y receptor proteins [Figure 26].

Example 4

Isolation of Mouse Pancreas-Derived G Protein Coupled Receptor Protein-Encoding DNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Mouse Pancreatic β -Cell Strain, MIN6 and Synthesis of cDNA

A total RNA was prepared from the mouse pancreatic β -cell strain, MIN6 (Jun-ichi Miyazaki et al., Endocrinology, Vol. 127, No. 1, p.126-132) according to the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J., 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with mouse Moloney Leukemia virus (MMLV) reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE buffer (10 mM Tris-HCl at pH8.0, 1 mM EDTA at pH8.0).

- 1 9 6 -

(2) Amplification of Receptor cDNA by PCR Using MIN6-Derived cDNA and Sequencing

By using, as a template, 5 μ l of cDNA prepared from the mouse pancreatic β -cell strain, MIN6 in the above 5 step (1), PCR amplification using the DNA primers synthesized in Example 1 was carried out under the same conditions as in Example 3(2). The resulting PCR product was 10 subcloned into the plasmid vector, pCRTM II, in the same manner as in Example 2 to obtain a plasmid, pG3-2. The plasmid pG3-2 15 was transfected into E. coli INV α F' to obtain transformed Escherichia coli INV α F'/pG3-2.

By using, as a template, 5 μ l of the cDNA prepared from the mouse pancreatic β -cell strain, MIN6, PCR amplification using DNA primers as disclosed in Libert F. et 15 al., "Science, 244:569-572, 1989", i.e., a degenerate synthetic primer represented by the following sequence:

5'-CTGTG (C or T) G (C or T) (G or C) AT (C or T) GCIIT
(G or T) GA (C or T) (A or C) G (G or C) TAC-3'

(SEQ ID NO: 60)

20 wherein I is inosine; and
a degenerate synthetic primer represented by the following sequence:

5'-A (G or T) G (A or T) AG (A or T) AGGGCAGCCAGCAGAI
(G or C) (A or G) (C or T) GAA-3'

(SEQ ID NO: 61)

25 wherein I is inosine,
was carried out under the same conditions as in Working Example 1. The resulting PCR product was subcloned into the plasmid vector, pCRTM II, in the same manner as described in 30 Example 3(2) to obtain a plasmid, pG1-10.

The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data

- 1 9 7 -

of the nucleotide sequence obtained were analyzed with DNASIS (Hitachi System Engineering Co., Japan).

Figure 27 shows a mouse pancreatic β -cell strain MIN6-derived G protein coupled receptor protein-encoding DNA and an amino acid sequence encoded by the isolated DNA based upon the nucleotide sequences of plasmids pG3-2 and pG1-10 which are held by the transformant Escherichia coli INV α F'/pG3-2. The underlined portions represent regions corresponding to the synthetic primers.

Homology retrieval was carried out based upon the determined nucleotide sequence [Figure 27]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment obtained. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence was converted into an amino acid sequence [Figure 27], hydrophobicity plotting was carried out to confirm the presence of six hydrophobic regions [Figure 28]. Upon comparing the amino acid sequence with that of p19P2 obtained in Example 3, furthermore, a high degree of homology was found as shown in [Figure 61]. As a result, it is strongly suggested that the G protein coupled receptor proteins encoded by pG3-2 and pG1-10 recognize the same ligand as the G protein coupled receptor protein encoded by p19P2 does while the animal species from which the receptor proteins encoded by pG3-2 and pG1-10 are derived is different from that from which the receptor protein encoded by p19P2 is.

Example 5

Isolation of Human Amygdaloid Nucleus-Derived G Protein Coupled Receptor Protein-Encoding DNA

(1) Amplification of Receptor cDNA by PCR Using Human Amygdaloid Nucleus-Derived cDNA

By using an amplified human amygdala-derived cDNA (QuickClone, CLONTECH Laboratories, Inc.) as a template, PCR amplification using the DNA primers synthesized in Example 1 was carried out. The composition of the reaction solution

- 1 9 8 -

consisted of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 1 μ M, 1 ng of the template cDNA, 0.25 mM dNTPs, 1 μ l of Tag DNA polymerase and a buffer attached to the enzyme kit, and the total amount of
5 the reaction solution was made to be 100 μ l. The cycle for amplification including 95 °C for 1 min., 55 °C for 1 min. and 72 °C for 1 min. was repeated 30 times by using a Thermal Cycler (Perkin-Elmer Co.). Prior to adding Tag DNA polymerase, the remaining reaction solution was mixed and was heated at 95 °C
10 for 5 minutes and at 65 °C for 5 minutes. The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(2) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding
15 Nucleotide Sequence of Inserted cDNA Region

The PCR products were separated by using a 0.8% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover
20 DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to the plasmid vector, pCRTM II. The recombinant vectors were introduced into E. coli INV α F' competent cells (Invitrogen Co.) to produce transformants. Then, transformant clones
25 having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia coli INV α F'/p63A2.

30 The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNA thus prepared was cut by EcoRI to confirm the size of the cDNA fragment
35 that was inserted. An aliquot of the remaining DNA was further processed with RNase, extracted with phenol/chloroform, and

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precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the 5 nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan).

Homology retrieval was carried out based upon the determined nucleotide sequences [Figures 29 and 30]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in 10 the plasmid, p63A2 possessed by the transformant Escherichia coli INV α F'/p63A2. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequences were converted into amino acid 15 sequences [Figures 29 and 30], and homology retrieval was carried out in view of hydrophobicity plotting [Figures 31 and 32] and at the amino acid sequence level to find homology relative to mouse GIR [Figure 33].

Example 6

20 Cloning of Human Pituitary Gland-Derived G Protein Coupled Receptor Protein cDNA

(1) Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Human Pituitary Gland-Derived cDNA Library

25 The DNA library constructed by Clontech Co. wherein λ gt11 phage vector is used (CLONTECH Laboratories, Inc.; CLH L1139b) was employed as a human pituitary gland-derived cDNA library. The human pituitary gland cDNA library (2×10^6 pfu (plaque forming units)) was mixed with 30 E. coli Y1090 treated with magnesium sulfate, and incubated at 37°C for 15 minutes followed by addition of 0.5% agarose (Pharmacia Co.) LB. The E. coli was plated onto a 1.5% agar (Wako-Junyaku Co.) LB plate (containing 50 μ g/ml of ampicillin). A nitrocellulose filter was placed on the plate 35 on which plaques were formed and the plaque was transferred onto the filter. The filter was denatured with an alkali and

- 2 0 0 -

then heated at 80 °C for 3 hours to fix DNAs.

The filter was incubated overnight at 42 °C together with the probe mentioned herein below in a buffer containing 50% formamide, 5 x SSPE (20 x SSPE (pH 7.4) is 3 M NaCl, 0.2 M NaH₂PO₄ · H₂O, 25 mM EDTA), 5 X Denhardt's solution (Nippon Gene, Japan), 0.1% SDS and 100μ g/ml of salmon sperm DNA for hybridization.

10 The probe used was obtained by cutting the DNA fragment inserted in the plasmid, p19P2, obtained in Working Example 3, with EcoRI, followed by recovery and labelling by incorporation of [³²P]dCTP (Dupont Co.) with a random prime DNA labelling kit (Amasham Co.).

15 It was washed with 2 x SSC (20 x SSC is 3 M NaCl, 0.3 M sodium citrate), 0.1% SDS at 55 °C for 1 hour and, then, subjected to an autoradiography at -80 °C to detect hybridized plaques.

20 In this screening, hybridization signals were recognized in three independent plaques. Each DNA was prepared from the three clones. The DNAs digested with EcoRI were subjected to an agarose electrophoresis and were analyzed by the southern blotting using the same probe as the one used in the screening. Hybridizing bands were identified at about 0.7kb, 0.8 kb and 2.0kb, respectively. Among them, the DNA fragment corresponding to the band at about 2.0kb (λ hGR3) 25 was selected. The λ hGR3-derived EcoRI fragment with a hybridizable size was subcloned to the EcoRI site of the plasmid, pUC18, and E. coli JM109 was transformed with the plasmid to obtain transformant E. coli JM109/phGR3. A restriction enzyme map of the plasmid, phGR3, was prepared 30 relying upon a restriction enzyme map deduced from the nucleotide sequence as shown in Example 3. As a result, it was learned that it carried a full-length receptor protein-encoding DNA which was predicted from the receptor protein-encoding DNA as shown in Example 3.

- 2 0 1 -

(2) Sequencing of Human Pituitary Gland-Derived Receptor Protein cDNA.

Among the EcoRI fragments inserted in the plasmid, phGR3, obtained in the above step (1), the from EcoRI to NheI 5 nucleotide sequence with about 1330bp that is considered to be a receptor protein-coding region was sequenced. Concretely speaking, by utilizing restriction enzyme sites that exist in the EcoRI fragments, unnecessary parts were removed or necessary fragments were subcloned in order to prepare 10 template plasmids for analyzing the nucleotide sequence.

The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data of 15 the nucleotide sequence obtained were analyzed with DNAsis (Hitachi System Engineering Co., Japan).

Figure 34 shows a nucleotide sequence of from 20 immediate after the EcoRI site up to the NheI site encoded by phGR3. The nucleotide sequence of the human pituitary gland-derived receptor protein-encoding DNA corresponds to the nucleotide sequence of from 118th to 123rd nucleotides [Figure 34]. An amino acid sequence of the receptor protein that is encoded by the nucleotide sequence is shown in 25 Figure 34. Figure 36 shows the results of hydrophobicity plotting based upon the amino acid sequence.

(3) Northern Hybridization with Human Pituitary Gland-Derived Receptor Protein-Encoding phGR3

Northern blotting was carried out in order to detect 30 the expression of phGR3-encoded human pituitary gland-derived receptor proteins in the pituitary gland at a mRNA level. Human pituitary gland mRNA (2.5 μ g, Clontech Co.) was used as a template mRNA and the same as the probe used in Working Example 5 was used as a probe. Nylon membrane (Pall Biodyne, U.S.A.) was used as a filter for northern blotting and 35 migration of the mRNA and adsorption (sucking) thereof with the blotting filter was carried out according to the method as

- 2 0 2 -

disclosed in Molecular Cloning, Cold Spring Harbor Laboratory Press, 1989.

The hybridization was effected by incubating the above-mentioned filter and probe in a buffer containing 50% formamide, 5 x SSPE, 5 X Denhardt's solution, 0.1% SDS and 100 μ g/ml of salmon sperm DNA overnight at 42 °C. The filter was washed with 0.1 x SSC, 0.1% SDS at 50 °C and, after drying with an air, was exposed to an X-ray film (XAR5, Kodak) for three days at -80 °C. The results were as shown in Figure 35 from which it is considered that the receptor gene encoded by phGR3 is expressed in the human pituitary gland.

Example 7

Cloning of Mouse Pancreatic β -Cell Strain, MIN6-Derived G

Protein Coupled Receptor Protein cDNA

15 (1) Preparation of Poly(A)⁺ RNA Fraction from Mouse Pancreatic β -Cell Strain, MIN6 and Synthesis of cDNA

A total RNA was prepared from the mouse pancreatic β -cell strain, MIN6 (Jun-ichi Miyazaki et al., Endocrinology, Vol. 127, No. 1, p.126-132) according to the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J., 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE.

30 (2) Amplification of Receptor cDNA by PCR Using MIN6-Derived cDNA and Sequencing

By using, as a template, 5 μ l of cDNA prepared from the mouse pancreatic β -cell strain, MIN6 in the above step (1), PCR amplification using the DNA primers synthesized 35 in Example 1 was carried out. A reaction solution was

- 2 0 3 -

composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM,
5 0.25 mM dNTPs, 1 μ l of Taq DNA polymerase and 10 μ l of 10 \times buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min.
10 and 60 °C for 3 min. was repeated 30 times by using a Thermal Cycler (Perkin-Elmer Co.). Prior to adding Taq DNA polymerase, the remaining reaction solution was mixed and was heated at 95 °C for 5 minutes and at 65 °C for 5 minutes. The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and
15 Selection of Novel Receptor Candidate Clone via Decoding
Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2)
were separated by using a 0.8% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and
20 precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to the plasmid vector, PCRTM II. The recombinant vectors were introduced into E. coli JM109 competent cells (Takara Shuzo Co., Japan) to
25 produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin, IPTG (isopropylthio- β -D-galactoside) and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain
30 transformant Escherichia coli JM109/p3H2-17.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared
35 was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was

- 2 0 4 -

further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a 5 fluorescent automatic sequencer, and the data of the nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan).

Homology retrieval was carried out based upon the determined nucleotide sequence [Figure 37]. As a result, it 10 was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli JM109/p3H2-17. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted 15 into an amino acid sequence [Figure 37], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 38] and at the amino acid sequence level to find homology relative to chicken ATP receptor (P34996), human somatostatin receptor subtype 3 (A46226), human somatostatin receptor subtype 4 (JN0605) and bovine neuropeptide Y receptor (S28787) 20 [Figure 39]. Abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers".

Example 8

25 Cloning of Mouse Pancreatic β -Cell Strain, MIN6-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Mouse Pancreatic β -Cell Strain, MIN6 and Synthesis of cDNA

A total RNA was prepared from the mouse pancreatic 30 β -cell strain, MIN6 (Jun-ichi Miyazaki et al., Endocrinology, Vol. 127, No. 1, p.126-132) according to the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J., 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g 35 of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected

- 2 0 5 -

to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in 5 ethanol, and was then dissolved in 30 μ l of TE.

(2) Amplification of Receptor cDNA by PCR Using MIN6-Derived CDNA and Sequencing

By using, as a template, 5 μ l of cDNA prepared from the mouse pancreatic β -cell strain, MIN6, in the above step (1), PCR amplification using the DNA primers synthesized in Example 1 was carried out. A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 10 0.25 mM dNTPs, 1 μ l of Tag DNA polymerase and 10 μ l of 10 \times buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 30 times by using a Thermal Cycler (Perkin-Elmer Co.). Prior to adding Tag DNA 15 polymerase, the remaining reaction solution was mixed and was heated at 95 °C for 5 minutes and at 65 °C for 5 minutes. The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and 25 Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2) were separated with a 0.8% low-melting temperature agarose 30 gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to the plasmid vector, PCRTM II. The recombinant vectors were introduced into 35 E. coli JM109 competent cells (Takara Shuzo Co., Japan)

to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin, IPTG and X-gal. Only transformant clones exhibiting white color were picked with 5 a sterilized toothstick to obtain transformant Escherichia coli JM109/p3H2-34.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to 10 prepare plasmid DNAs. An aliquot of the DNAs thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing 15 was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan).

Homology retrieval was carried out based upon the determined nucleotide sequence [Figure 40]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli 20 JM109/p3H2-34. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted into an amino acid sequence [Figure 40], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 41] and at the amino acid sequence level to 25 find homology relative to human somatostatin receptor subtype 2 (B41795) and rat-derived ligand unknown receptor (A39297) [Figure 42]. Abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers" 30 or "Entry Names".

Example 9

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Rabbit

5 Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

A total RNA was prepared from rabbit gastropyloric part smooth muscles by the guanidine thiocyanate method

(Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and,

then, poly(A)⁺ RNA fractions were prepared with a mRNA

10 purifying kit (Pharmacia Co.). Next, to 5 μ g of the

⁺ poly(A) RNA fraction was added a random DNA hexamer

(BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.)

in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product

was extracted with phenol/chloroform (1:1), precipitated

ethanol, and was then dissolved in 30 μ l of TE (Tris-EDTA solution).

(2) Amplification of Receptor cDNA by PCR Using Rabbit Gastropyloric Part Smooth Muscle-Derived cDNA and Sequencing

By using, as a template, 1 μ l of cDNA prepared from the rabbit gastropyloric part smooth muscle in the above step (1), PCR amplification using the DNA primers synthesized in Example 1 was carried out. A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1 μ l of Tag DNA polymerase and 10 μ l of buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 25 times by using a Thermal Cycler (Perkin-Elmer Co.). The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and Selection of Novel Receptor Candidate Clone via Decoding Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2) were separated with a 1.0% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to the plasmid vector, pCRTM II. The recombinant vectors were introduced into E. coli JM109 competent cells (Takara Shuzo Co., Japan) to produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin, IPTG and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia coli JM109/pMD4.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan). The determined nucleotide sequence was as shown in Figure 43. It was learned from Figure 43 that the cloned cDNA fragment was amplified from both sides with only the synthetic DNA primer having a nucleotide sequence represented by SEQ ID NO: 1 as synthesized in Example 1.

Homology retrieval was carried out based upon the

- 2 0 9 -

determined nucleotide sequence [Figure 43]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli JM109/pMD4.

5 To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted into an amino acid sequence [Figure 43], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 44] and at the amino acid sequence level to find homology relative
10 to rat ligand-unknown receptor protein (A35639) [Figure 45]. Abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers".

Example 10

15 Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Mouse Pancreatic β -Cell Strain, MIN6-Derived cDNA Library

(1) Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Mouse Pancreatic β -Cell Strain,
20 MIN6-Derived cDNA Library

TM Superscript Lambda System (BRL, Cat. 8256) distributed by BRL Co. and Gigapack II Gold (Stratagene, Cat. 200215) distributed by Stratagene Co. were used to construct MIN6-derived cDNA libraries. By using the above
25 kits, a MIN6 cDNA library with 2.2×10^6 pfu (plaque forming units) was constructed from 10μ g of MIN6 poly(A)⁺ RNA. The cDNA library was mixed with E. coli Y1090⁻ treated with magnesium sulfate, and incubated at 37 °C for 15 minutes followed by addition of 0.5% agarose (Pharmacia Co.) LB.

30 The E. coli was plated onto a 1.5% agar (Wako-Junyaku Co.) LB plate (containing 50 μ g/ml of ampicillin). A nitrocellulose filter was placed on the plate on which plaques were formed and the plaque was transferred onto the filter. The filter was denatured with an alkali and then heated at 80 °C for 3 hours
35 to fix DNAs.

The filter was incubated overnight at 42 °C together

- 2 1 0 -

with the probe mentioned herein below in a buffer containing 50% formamide, 5 x SSPE, 5 X Denhardt's solution, 0.1% SDS and 100 μ g/ml of salmon sperm DNA for hybridization.

5 The probe used was obtained by cutting the DNA fragment inserted in the plasmid, p3H2-34, obtained in Working Example 8, with EcoRI, followed by recovery and labeling by incorporation of [32 P]dCTP (Dupont Co.) with a random prime DNA labelling kit (Amasham Co.).

10 It was washed with 2 x SSC (150 mM NaCl and 15 mM sodium citrate), 0.1% SDS at 55 °C for 1 hour and, then, subjected to an autoradiography at -80 °C to detect hybridized plaques.

15 In this screening, hybridization signals were recognized in two independent plaques. Each DNA was prepared from the two clones. The DNAs digested with SalI and NotI were subjected to an agarose electrophoresis and were analyzed. Inserted fragments were identified at about 2.0kb and 3.0kb, respectively. Between them, the DNA fragment corresponding to the band at about 3.0kb (λ No.20) 20 was selected. The λ No.20-derived NotI-SalI fragment with about 3.0kb was subcloned into the NotI-SalI site of the plasmid, pBluescriptTM II SK(+), and E. coli JM109 was transformed with the plasmid to obtain a transformant E. coli JM109/pMGR20. A restriction enzyme map of the 25 plasmid, pMGR20, was prepared relying upon a restriction enzyme map deduced from the nucleotide sequence as shown in Working Example 8. As a result, it was learned that it carried a full-length receptor protein-encoding DNA which was predicted from the receptor protein-encoding DNA as shown in Working 30 Example 8.

(2) Sequencing of MIN6-Derived Receptor Protein Full-Length cDNA

35 Among the NotI-SalI fragments inserted in the plasmid, pMGR20, obtained in the above step (1), the nucleotide sequence with total 1607bp, including not only a region that is considered to be a receptor protein-coding region (ORF) but

- 2 1 1 -

also a neighboring region thereof was sequenced. Concretely speaking, by utilizing restriction enzyme sites that exist in the NotI-SalI fragments, unnecessary parts were removed or necessary fragments were subcloned in order to prepare
5 template plasmids for analyzing the nucleotide sequence thereof. As for the nucleotide sequences of part of the regions, primers for sequencing were synthesized based upon the nucleotide sequences that were determined already and used to make confirmation.
10 The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with the fluorescent automatic sequencer (ABI Co.), and the data of the nucleotide sequence obtained were analyzed with DNASIS
15 (Hitachi System Engineering Co., Japan).

Figure 46 shows a nucleotide sequence around an open reading frame (ORF) of a mouse galanin receptor protein encoded by the cDNA insert in pMGR20. The nucleotide sequence of mouse galanin receptor protein-encoding DNA corresponds to from the 481st to 1525th nucleotides of the nucleotide sequence in Figure 46. The nucleotide sequence was converted into an amino acid sequence [Figure 46] and hydrophobicity plotting was carried out [Figure 47]. Since the amino acid sequence [Figure 46] has 92% homology to the human-derived galanin receptor protein at the amino acid sequence level [Figure 48], it was learned that the cDNA insert in the pMGR20 is a mouse-derived galanin receptor protein-encoding cDNA.
20
25

Example 11

30 Preparation of Synthetic DNA Primer for Amplifying G Protein Coupled Receptor Protein-Encoding DNA

Highly homologous parts were found by comparing nucleotide sequences corresponding to or near the third membrane-spanning domain [3C and 3D in Figure 4] and the sixth
35 membrane-spanning domain [6C of Figure 6] among known G protein coupled receptors, i.e., rat-derived angiotensin II

- 2 1 2 -

receptor protein (L32840), rat-derived angiotensin Ib receptor protein (X64052), rat-derived angiotensin receptor protein subtype (M90065), human-derived angiotensin Ia receptor protein (M91464), rat-derived cholecystokinin_A receptor protein (M88096), rat-derived cholecystokinin_B receptor protein (M99418), human-derived cholecystokinin_B receptor protein (L04473), mouse-derived low-affinity interleukin 8 receptor protein (M73969), human-derived high-affinity interleukin 8 receptor protein (X65858), mouse-derived C5a anaphylatoxin receptor protein (S46665), human-derived N-formyl peptide receptor protein (M60626), etc.

The aforementioned abbreviations in parentheses are reference numbers that are indicated when the GenBank/EMBL data base is retrieved, and are usually called "Accession Numbers".

It was planned to incorporate mixed bases relying upon the base regions that were in agreement with a large number of receptor protein cDNAs in order to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions. Based upon these sequences, the degenerate synthetic DNA (3D of Figure 4) having a nucleotide sequence represented by SEQ ID NO: 3 which is complementary to the homologous nucleotide sequence of Figure 4 and the degenerate synthetic DNA (nucleotide sequence complementary to 6C of Figure 6) having a nucleotide sequence represented by SEQ ID NO: 4 were produced. Nucleotide synthesis was carried out by a DNA synthesizer.

[Synthetic DNAs]

5'-CTCGC (G or C) GC (C or T) (A or C) TI (A or G) G
30 (C or T) ATGGA (C or T) CGITAT-3'
(SEQ ID NO:3)

5'-CATGT (A or G) G (T or A) AGGGAAICCAG (G or C) A
(A or C) AI (A or G) A (A or G)(A or G) AA-3'
(SEQ ID NO:4)

- 2 1 3 -

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

Example 12

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein cDNA

10 (1) Preparation of Poly(A)⁺ RNA Fraction from Rabbit Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

A total RNA was prepared from rabbit gastropyrolic part smooth muscles by the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, 15 then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.)

20 in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE.

25 (2) Amplification of Receptor cDNA by PCR Using Rabbit Gastropyrolic Part Smooth Muscle-Derived cDNA and Sequencing

30 By using, as a template, 1 μ l of cDNA prepared from the rabbit gastropyrolic part smooth muscle in the above step (1), PCR amplification using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 3 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 4 synthesized in Example 11 was carried out.

35 A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1 μ l of Tag DNA polymerase

and 10 μ l of buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 25 times by using 5 a Thermal Cycler (Perkin-Elmer Co.). The amplified products were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and
Selection of Novel Receptor Candidate Clone via Decoding
10 Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2) were separated with a 1.0% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were heat-melted, extracted with phenol and 15 precipitated in ethanol to recover DNAs. According to the protocol attached to a TA Cloning Kit (Invitrogen Co.), the recovered DNAs were subcloned to the plasmid vector, pPCRTM II. The recombinant vectors were introduced into E. coli JM109 competent cells (Takara Shuzo Co., Japan) to 20 produce transformants. Then, transformant clones having a cDNA-inserted fragment were selected in an LB agar culture medium containing ampicillin, IPTG and X-gal. Only transformant clones exhibiting white color were picked with a sterilized toothstick to obtain transformant Escherichia 25 coli JM109/pMJ10.

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with an automatic plasmid extracting machine (Kurabo Co., Japan) to 30 prepare plasmid DNAs. An aliquot of the DNAs thus prepared was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing 35 was carried out by using a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer, and the data of the

- 2 1 5 -

nucleotide sequences obtained were read by using DNASIS (Hitachi System Engineering Co., Japan). The determined nucleotide sequence was as shown in Figure 49.

Homology retrieval was carried out based upon the 5 determined nucleotide sequence [Figure 49]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli JM109/pMJ10. To further confirm this fact, by using DNASIS 10 (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted into an amino acid sequence [Figure 49], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 50] and at the amino acid sequence level to find homology relative to human ligand unknown receptor protein 15 (B42009), human N-formyl peptide receptor protein (JC2014), rabbit N-formyl peptide receptor protein (A46520), mouse C5a anaphylatoxin receptor protein (A46525) and bovine neuropeptide Y receptor protein (S28787) [Figure 51]. Abbreviations in parentheses are reference numbers assigned when they are 20 registered as data to NBRF-PIR/Swiss-PROT and are usually called "Accession Numbers".

Example 13

Preparation of Synthetic DNA Primer for Amplifying G Protein Coupled Receptor Protein-Encoding DNA

A comparison of nucleotide sequences coding for 25 regions corresponding to or near the third membrane-spanning domain among known G protein coupled receptors, i.e., mouse-derived κ -opioid receptor protein (L11064), mouse-derived δ -opioid receptor protein (L11065), rat-derived μ -opioid receptor protein (D16349), mouse-derived bradykinin B2 receptor protein (X69676), rat-derived bradykinin B2 receptor protein (M599967), mouse-derived bombesin receptor protein (M35328), human-derived neuromedin B receptor protein (M73482), human-derived gastrin releasing peptide receptor protein (M73481), human-derived bombesin receptor protein subtype 3 (L08893), mouse-derived substance K receptor protein 30 35

- 2 1 6 -

(X62933), mouse-derived substance P receptor protein (X62934), rat-derived neurokinin 3 receptor protein (J05189), rat-derived endothelin receptor protein (M60786), rat-derived ligand unknown receptor proteins (L04672), (X61496), (X59249) and (L09249), mouse-derived ligand unknown receptor protein (P30731), human-derived ligand unknown receptor proteins (M31210) and (U03642), etc. was made. In particular, the degenerate DNA primer having a nucleotide sequence (3B in Figure 3; SEQ ID NO: 6) with highly common bases (highly homologous nucleotides) was synthesized to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions on the basis of nucleotide sequence regions that were in agreement with a large number of receptor cDNAs. Nucleotide synthesis was carried out by a DNA synthesizer.

The nucleotide sequence represented by SEQ ID NO: 6 is:
5'-CTGAC (C or T) G (C or T) TCTI (A or G)(G or C) I
(A or G)(C or T) TGAC (A or C) G (A, C or G) TAT-3'

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

Furthermore, a comparison of nucleotide sequences coding for regions corresponding to or near the sixth membrane-spanning domain among known G protein coupled receptors, i.e., mouse-derived κ -opioid receptor protein (L11064), mouse-derived δ -opioid receptor protein (L11065), rat-derived μ -opioid receptor protein (D16349), mouse-derived bradykinin B2 receptor protein (X69676), rat-derived bradykinin B2 receptor protein (M59967), mouse-derived bombesin receptor protein (M35328), human-derived neuromedin B receptor protein

- 2 1 7 -

(M73482), human-derived gastrin releasing peptide receptor protein (M73481), human-derived bombesin receptor protein subtype 3 (L08893), mouse-derived substance K receptor protein (X62933), mouse-derived substance P receptor protein (X62934),
5 rat-derived neurokinin 3 receptor protein (J05189), rat-derived endothelin receptor protein (M60786), rat-derived ligand unknown receptor proteins (L04672), (X61496), (X59249) and (L09249), mouse-derived ligand unknown receptor protein (P30731), human-derived ligand unknown receptor proteins
10 (M31210) and (U03642), etc. was made. In particular, the degenerate DNA primer having a nucleotide sequence (SEQ ID NO: 8) which is complementary to the nucleotide sequence (6A in Figure 5) with highly common bases (highly homologous nucleotides) was synthesized to enhance base
15 agreement of sequences with as many receptor cDNAs as possible even in other portions on the basis of base regions that are in agreement with a large number of receptor cDNAs.

The nucleotide sequence represented by SEQ ID NO: 8 is:

20 5'-GATGTG (A or G) TA (A or G) GG (G or C)(A or G)
ICCAACAGAIG (A or G) (C or T) AAA-3'

The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in
25 parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

The aforementioned abbreviations in parentheses are reference numbers indicated when the GenBank/EMBL data base is
30 retrieved and are usually called "Accession Numbers".

- 2 1 8 -

Example 14

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Rabbit

5 Gastropyrolic Part Smooth Muscle and Synthesis of cDNA
A total RNA was prepared from rabbit gastropyrolic part smooth muscles by the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the 10 poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit 15 to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE.

(2) Amplification of Receptor cDNA by PCR Using Rabbit
Gastropyrolic Part Smooth Muscle-Derived cDNA and
20 Sequencing

By using, as a template, 1 μ l of cDNA prepared from the rabbit gastropyrolic part smooth muscle in the above step (1), PCR amplification using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 6 and the DNA 25 primer having a nucleotide sequence represented by SEQ ID NO: 8 synthesized in Example 13 was carried out. A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1 μ l of Tag DNA polymerase 30 and 10 μ l of buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 25 times by using a Thermal Cycler (Perkin-Eimer Co.). The amplified products 35 were confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

(3) Subcloning of PCR Product into Plasmid Vector and
Selection of Novel Receptor Candidate Clone via Decoding
Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2)
5 were separated by using a 1.0% low-melting temperature agarose
gel, the band parts were excised from the gel with a razor
blade, and were heat-melted, extracted with phenol and
precipitated in ethanol to recover DNAs. According to the
protocol attached to a TA Cloning Kit (Invitrogen Co.),
10 the recovered DNAs were subcloned to the plasmid vector,
pCRTM II. The recombinant vectors were introduced into
E. coli JM109 competent cells (Takara Shuzo Co., Japan) to
produce transformants. Then, transformant clones having
15 a cDNA-inserted fragment were selected in an LB agar
culture medium containing ampicillin, IPTG and X-gal. Only
transformant clones exhibiting white color were picked with
a sterilized toothstick to obtain transformant Escherichia
20 coli JM109/pMH28.

The individual clones were cultured overnight in an
LB culture medium containing ampicillin and treated with an
automatic plasmid extracting machine (Kurabo Co., Japan) to
prepare plasmid DNAs. An aliquot of the DNAs thus prepared
was cut by EcoRI to confirm the size of the cDNA fragment
that was inserted. An aliquot of the remaining DNAs was
25 further processed with RNase, extracted with phenol/chloroform,
and precipitated in ethanol so as to be condensed. Sequencing
was carried out by using a DyeDeoxy terminator cycle
sequencing kit (ABI Co.), the DNAs were decoded by using a
fluorescent automatic sequencer, and the data of the
30 nucleotide sequences obtained were read by using DNASIS
(Hitachi System Engineering Co., Japan). The determined
nucleotide sequence was as shown in Figure 52.

Homology retrieval was carried out based upon the
determined nucleotide sequence [Figure 52]. As a result,
35 it was learned that a novel G protein coupled receptor
protein was encoded by the cDNA fragment insert in the
plasmid possessed by the transformant Escherichia coli

- 2 2 0 -

JM109/pMH28. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the nucleotide sequence were converted into an amino acid sequence [Figure 52], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 53] and at the amino acid sequence level to find homology relative to mouse IL-8 receptor protein (P35343), human somatostatin receptor protein 1 (A41795) and human somatostatin receptor protein 4 (A47457)[Figure 54].
5 The aforementioned abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR or SWISS-PROT and are usually called "Accession Numbers".
10

Example 15

Preparation of Synthetic DNA Primer for Amplifying G Protein Coupled Receptor Protein-Encoding DNA

15 A comparison of nucleotide sequences coding for regions corresponding to or near the second membrane-spanning domain among known G protein coupled receptors, i.e., human-derived galanin receptor (HUMGALAREC), rat-derived α -1B-adrenergic receptor (RATADR1B), human-derived
20 β -1-adrenergic receptor (HUMADRB1), rabbit-derived IL-8 receptor (RABIL8RSB), human-derived opioid receptor (HUMOPIODRE), bovine-derived substance K receptor (BTSKR), human-derived somatostatin receptor-2 (HUMSTR12A), human-derived somatostatin receptor-3 (HUMSSTR3Y),
25 human-derived gastrin receptor (HUMGARE), human-derived cholecystokinin A receptor (HUMCCKAR), human-derived dopamine receptor-D5 (HUMD1B), human-derived serotonin receptor 5HT1E (HUM5HT1E), human-derived dopamine receptor D4 (HUMD4C), mouse-derived serotonin receptor-2 (MMSERO), rat-derived
30 α -1A-adrenergic receptor (RATADRA1A), rat-derived histamine H2 receptor (S57565), etc. was made. In particular, the degenerate DNA primer having a nucleotide sequence (T2A in Figure 7, SEQ ID NO: 10) with highly common bases (highly homologous nucleotides) was synthesized to enhance base
35 agreement of sequences with as many receptor cDNAs as possible even in other regions on the basis of nucleotide sequence

- 2 2 1 -

regions that were in agreement with a large number of receptor cDNAs. Nucleotide synthesis was carried out by a DNA synthesizer.

The nucleotide sequence represented by SEQ ID NO: 10
5 is:
5'-GYCACCAACN₂WSTTCATCCTSWN₂HCTG-3'

wherein S represents G or C; Y represents C or T; W represents A or T; H represents A, C or T and N₂ represents I.

10 The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis, provided that I denotes inosine.

15 Furthermore, a comparison of nucleotide sequences coding for regions corresponding to or near the seventh membrane-spanning domain among known G protein coupled receptors, i.e., human-derived galanin receptor (HUMGALAREC), rat-derived A1 adenosine receptor (RAT1ADREC), porcine-derived angiotensin receptor (PIGA2R), rat-derived serotonin receptor (RAT5HTRTC), human-derived dopamine receptor (S58541), human-derived gastrin releasing peptide receptor (HUMGRPR), mouse-derived GRP/bombesin receptor (MUSGRPBM), rat-derived vascular type 1 angiotensin receptor (RRVT1AIIR),
20 human-derived muscarinic acetylcholine receptor (HSHM4), human-derived β -1 adrenergic receptor (HUMDRB1), human-derived gastrin receptor (HUMGARE), rat-derived cholecystokinin receptor (RATCCKAR), rat-derived ligand unknown receptor (S59748), human-derived somatostatin receptor (HUMSST28A), rat-derived ligand unknown receptor (RNGPROCR), mouse-derived somatostatin receptor 1 (MUSSRI1A), human-derived α -A1-adrenergic receptor (HUMA1AADR), mouse-derived delta-opioid receptor (S66181), human-derived somatostatin
25
30

- 2 2 -

receptor-3 (HUMSSTR3Y), etc. was made. In particular, the degenerate DNA primer having a nucleotide sequence (T7A in Figure 8, SEQ ID NO: 11) with highly common bases (highly homologous nucleotides) was synthesized to enhance base agreement of sequences with as many receptor cDNAs as possible even in other regions on the basis of nucleotide sequence regions that were in agreement with a large number of receptor cDNAs. Nucleotide synthesis was carried out by a DNA synthesizer.

10 The nucleotide sequence represented by SEQ ID NO: 11 is:

5'-ASN₂SAN₂RAAGSARTAGAN₂GAN₂GGGRTT-3'

wherein R represents A or G; S represents G or C and N₂ represents I.

15 The parentheses indicate the incorporation of a plurality of bases, leading to multiple oligonucleotides in the primer preparation. In other words, nucleotide residues in parentheses of the aforementioned DNAs were incorporated in the presence of a mixture of plural bases at the time of synthesis,
20 provided that I denotes inosine.

The aforementioned abbreviations in parentheses are reference numbers indicated when the GenBank/EMBL data base is retrieved and are usually called "Accession Numbers".

Example 16

25 Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Rabbit

Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

A total RNA was prepared from rabbit gastropyrolic
30 part smooth muscles by the guanidine thiocyanate method
(Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and,
then, poly(A)⁺ RNA fractions were prepared with a mRNA
purifying kit (Pharmacia Co.). Next, to 5 μg of the

- 2 2 3 -

poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit 5 to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE.

10 (2) Amplification of Receptor cDNA by PCR Using Rabbit
Gastropyrolic Part Smooth Muscle-Derived cDNA and
Sequencing

By using, as a template, 1 μ l of cDNA prepared from the rabbit gastropyrolic part smooth muscle in the above step (1), PCR amplification using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 10 and the DNA 15 primer having a nucleotide sequence represented by SEQ ID NO: 11 synthesized in Example 15 was carried out. A reaction solution was composed of the synthetic DNA primers (SEQ: 5' primer sequence and 3' primer sequence) each in an amount of 100 pM, 0.25 mM dNTPs, 1 μ l of Tag DNA polymerase 20 and 10 μ l of buffer attached to the enzyme kit, and the total amount of the reaction solution was made to be 100 μ l. The cycle for amplification including 96 °C for 30 sec., 45 °C for 1 min. and 60 °C for 3 min. was repeated 25 times with a Thermal Cycler (Perkin-Elmer Co.). The amplified products were 25 confirmed relying upon 1.2% agarose gel electrophoresis and ethidium bromide staining.

30 (3) Subcloning of PCR Product into Plasmid Vector and
Selection of Novel Receptor Candidate Clone via Decoding
Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2) were separated with a 1.4% low-melting temperature agarose gel, the band parts were excised from the gel with a razor blade, and were eluted electrophoretically, extracted with phenol and precipitated in ethanol to recover DNAs. According to the 35 protocol attached to a TA Cloning Kit (Invitrogen Co.),

the recovered DNAs were subcloned to the plasmid vector,
pPCRTM II. The recombinant vectors were introduced into
E. coli JM109 competent cells (Takara Shuzo Co., Japan) to
produce transformants. Then, transformant clones having
5 a cDNA-inserted fragment were selected in an LB agar
culture medium containing ampicillin, IPTG and X-gal. Only
transformant clones exhibiting white color were picked with
a sterilized toothstick to obtain 100 transformant clones.

The individual clones were cultured overnight in an
10 LB culture medium containing ampicillin and treated with the
automatic plasmid extracting machine PI-100 (Kurabo Co., Japan)
to prepare plasmid DNAs. An aliquot of the DNA thus prepared
was cut by EcoRI to confirm the size of the cDNA fragment
that was inserted. An aliquot of the remaining DNA was
15 further processed with RNase, extracted with phenol/chloroform,
and precipitated in ethanol so as to be condensed. Sequencing
was carried out by using a DyeDeoxy terminator cycle
sequencing kit (ABI Co.), the DNAs were decoded by using a
fluorescent automatic sequencer.

20 Homology retrieval was carried out based upon the
determined nucleotide sequence by using DNASIS (Hitachi System
Engineering Co., Japan). As a result, it was learned that a
novel G protein coupled receptor protein was encoded by
the cDNA fragment insert in the plasmid possessed by
25 the transformant Escherichia coli JM109/pMN7. Figure 56 and
Figure 56 show the nucleotide sequences of the cDNA fragments.
To further confirm this fact, by using DNASIS (Hitachi System
Engineering Co., Japan), the nucleotide sequences were
converted into amino acid sequences [Figure 55] and [Figure 56],
30 and hydrophobicity plotting was carried out [Figure 57].
As a result, the presence of hydrophobic domains which prove
that it is a G protein coupled receptor protein were confirmed.
Furthermore, homology retrieval was carried out at the amino
acid sequence level to find that the DNAs were novel receptor
35 proteins having 27% homology relative to rat-derived
 β_3 -adrenaline receptor protein (A41679), 29% homology relative
to rat-derived serotonin (5-HT6) receptor protein (JN0591),

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27% homology relative to dog-derived histamine H₂ receptor protein (A39008), 27% homology relative to human-derived somatostatin receptor (type 4) protein (JN0605), 24% homology relative to human-derived dopamine D₁ receptor protein (S11377), 23% homology relative to rat-derived neuropeptide Y receptor protein (JH0164), 31% homology relative to human-derived cholecystokinin B receptor protein (JC1352), and 30% homology relative to rat-derived gastrin receptor protein (JQ1614). The aforementioned abbreviations in parentheses are reference numbers assigned when they are registered as data to NBRF-PIR and are usually called "Accession Numbers".

Example 17

Amplification of Receptor cDNA by PCR Using MIN6-Derived cDNA and Sequencing

By using, as a template, 5 μl of cDNA prepared from the mouse pancreatic β-cell strain, MIN6 in Working Example 4 (1), PCR amplification using the DNA primers synthesized in Example 4 (2) as disclosed in Libert F. et al., "Science, 244:569-572, 1989", i.e., a synthetic primer represented by the following sequence:

5'-CTGTG (C or T) G (C or T) (G or C) AT (C or T) GCIIT
(G or T) GA (C or T) (A or C) G (G or C) TAC-3'
(SEQ ID NO: 60)

wherein I is inosine; and
a synthetic primer represented by the following sequence:

5'-A (G or T) G (A or T) AG (A or T) AGGGCAGCCAGCAGAI
(G or C) (A or G) (C or T) GAA-3'
(SEQ ID NO: 61)

wherein I is inosine, was carried out under the same conditions as in Example 3 (1). The resulting PCR product was subcloned to the plasmid vector, pCRTM II, in the same manner as in Example 3 (2) to obtain a plasmid, p5S38. The plasmid p5S38 was transfected into E. coli JM109 to obtain

transformant Escherichia coli JM109/p5S38.

The reaction for determining the nucleotide sequence (sequencing) was carried out with a DyeDeoxy terminator cycle sequencing kit (ABI Co.), the DNA was decoded with 5 the fluorescent automatic sequencer (ABI Co.), and the data of the nucleotide sequence obtained were read with DNASIS (Hitachi System Engineering Co., Japan).

Figure 62 shows a mouse pancreatic β -cell strain MIN6-derived G protein coupled receptor protein-encoding cDNA 10 (SEQ ID NO: 33) and an amino acid sequence (SEQ ID NO: 28) encoded by the isolated DNA based upon the nucleotide sequence of plasmid, p5S38. The underlined portions represent regions corresponding to the synthetic primers.

Homology retrieval was carried out based upon the 15 determined nucleotide sequence [Figure 62]. As a result, it was learned that a novel G protein coupled receptor protein was encoded by the cDNA fragment obtained. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan), the nucleotide sequence was converted into an 20 amino acid sequence [Figure 62], and hydrophobicity plotting was carried out to confirm the presence of four hydrophobic regions [Figure 64]. Upon comparing the amino acid sequence with those encoded by p19P2 obtained in Example 3 (2) and encoded by pG3-2 obtained in Example 4 (2), furthermore, 25 a high degree of homology was found as shown in Figure 63. As a result, it is strongly suggested that the mouse pancreatic β -cell strain, MIN6-derived G protein coupled receptor protein encoded by p5S38 recognizes the same ligand as the human pituitary gland-derived G protein coupled receptor 30 protein encoded by p19P2 does while the animal species from which the receptor protein encoded by p5S38 is derived is different from that from which the receptor protein encoded by p19P2 is. It is also strongly suggested that the mouse pancreatic β -cell strain, MIN6-derived G protein coupled receptor protein encoded by p5S38 recognizes the same ligand as the mouse pancreatic β -cell strain, MIN6-derived G protein 35 coupled receptor proteins encoded by pG3-2 and pG1-10 do and

they are analogous receptor proteins one another (so-called "subtype").

Example 18

Northern Hybridization with cDNA Fragment Included in
5 MIN6-Derived Receptor Protein-Encoding p3H2-17

Mouse cell line, MIN6, Neuro-2a, poly(A)⁺RNA (2.5 μ g) and mouse brain, spleen, thymus and pancreas poly(A)⁺RNAs (2.5 μ g) were used as poly(A) RNAs. The DNA fragment inserted into the plasmid, p3H2-17, obtained in Example 7 (3) was recovered as a DNA fragment with about 400bp by cutting the plasmid with EcoRI and the resulting DNA fragment was labeled by incorporation of [³²P]dCTP (Dupont Co.) with a random prime DNA labeling kit (Amasham Co.). The about 400bp labeled DNA fragment was used as a probe for hybridization.

15 Nylon membrane (PALL Biodyne, U.S.A.) was used as a filter for northern blotting and migration of the poly(A)⁺RNA and adsorption (sucking) thereof with the blotting filter was carried out according to the method as disclosed in Molecular Cloning, Cold Spring Harbor Laboratory Press, 1989.

20 The hybridization was carried out by incubating the above-mentioned filter and probe in a buffer containing 50% formamide, 5 x SSPE (20 x SSPE (pH 7.4) is 3 M NaCl, 0.2 M NaH₂PO₄·H₂O, 25 mM EDTA), 5 X Denhardt's solution (Nippon Gene, Japan), 0.1% SDS and 100 μ g/ml of salmon sperm DNA overnight at 42 °C. The filter was washed with 0.1 x SSC (20 x SSC is 3 M NaCl, 0.3 M sodium citrate), 0.1% SDS at 50 °C and, after drying with an air, was exposed to an X-ray film (XAR5, Kodak) for 15 days at -80 °C. The results were as shown in Figure 65.

30 It is considered from Figure 65 that mRNA for the receptor gene encoded by the cDNA fragment included in p3H2-17 is expressed in the cell line, MIN6, Neuro-2a, and the mouse brain, pancreas, spleen and thymus and especially expressed in the mouse spleen and thymus intensely. The MIN6 signal position hybridizable in the northern hybridization plotting is different from those of other organ cells.

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Example 19

PCR Cloning of cDNA Comprising Whole Coding Regions of
Receptor Proteins from Mouse Spleen, Thymus-Derived
Poly(A)⁺ RNA and Sequencing

5 (1) PCR Cloning of cDNA Comprising Whole Coding Region of
Receptor Protein

In order to obtain a full-length open reading frame
(coding region) of the receptor protein encoded by the cDNA
fragment included in p3H2-17, PCR amplification was carried out
10 by 5'RACE and 3'RACE wherein poly(A)⁺ RNA derived from mouse
spleen and thymus was used.

Based on the nucleotide sequence of 3H2-17 which was
disclosed, the following 4 primers were synthesized:

(Nucleotide sequence of synthesized primer)

15 ① 5'-TAGTGTGTGGAGTCGTGTGGCTGGCTG-3'
(SEQ ID NO: 20)

② 5'-AGTCTTGCTGCCACAGGCATCCAGCG-3'
(SEQ ID NO: 21)

③ 5'-CAAGCCAGTAAGGCTATGAAGGGCAGCAAG-3'
(SEQ ID NO: 22)

20 ④ 5'-ACAGGACCTGCTGGGCCATCCTGGCGACACA-3'
(SEQ ID NO: 23)

The 5'RACE was carried out according to the protocol
of 5'Ampli Finder RACE kit from ClonTech Co. (ClonTech Co.).

25 In an embodiment, cDNA was prepared from 2 μ g each
of poly(A)⁺ RNAs derived from mouse spleen and thymus by using
the aforementioned primer ④ and ligated with an anchor
attached to the 5'RACE kit. A mixture of a 1/200 amount of the
cDNA thus prepared, the anchor and the aforementioned primer
30 ③ was subjected to PCR using 4 polymerases, Tag (Takara, Japan),
Ex Tag (Takara, Japan), Vent (New England Biolabs) and Pfu
(Stratagene) under the following conditions: 96 °C for 30 sec.,
60 °C for 60 sec., 72 °C for 90 sec. and 35 cycles. A 1/5
amount of the PCR product was subjected to agarose
35 electrophoresis and stained with ethidium bromide (EtBr).
The results are shown in Figure 66. The amplified DNA band

appeared at an about 1 kbp position and the isolated about 1 kbp DNA band which was synthesized from poly(A)⁺ RNAs derived from mouse spleen and thymus by the 5'RACE using Ex Tag polymerase was treated with SUPRECTM-01 (Takara, Japan) 5 to recover cDNA.

The isolated DNA was subcloned into pCRTM II vector by using a TA Cloning Kit (Invitrogen Co.) and the vector was transfected into E. coli JM109 to obtain 3 transformant clones, N26, N64 and N75. The clone, N26, holds the thymus-derived cDNA which is amplified by the 5'RACE and the clone, N75, holds the spleen-derived cDNA which is amplified by the 5'RACE (Figure 68). 10

The 3'RACE was carried out according to the protocol of 3' RACE kit (GIBCO BRL Co.).

In an embodiment, cDNA was prepared from 1 μ g each 15 of poly(A)⁺ RNAs derived from mouse spleen and thymus by using an adaptor primer attached to the 3' RACE kit. A mixture of the adaptor primer thus prepared and a 1/10 amount of cDNA which was prepared by using the aforementioned primer ① was subjected to 1st PCR using 4 polymerases, Taq (Takara, Japan), Ex Tag (Takara, Japan), Vent (NEB) and Pfu (Stratagene) under the following conditions: 96 °C for 30 sec., 55 °C for 60 sec., 72 °C for 120 sec. and 30 cycles. A mixture of a 1/50 amount of the 1st PCR product, the aforementioned primer ② 20 and the adaptor primer was subjected to 2nd PCR using the aforementioned polymerases under the same conditions as aforementioned herein in the 5'RACE process. A 1/5 amount of the 2nd PCR product was subjected to agarose electrophoresis and stained with ethidium bromide. The results are shown in 25 Figure 67. 30

The amplified DNA band appeared at an about 1 kbp position (which was synthesized from poly(A)⁺ RNAs derived from mouse thymus by the 3'RACE using Vent polymerase) and the amplified DNA band appeared at an about 1 kbp position (which 35 was synthesized from poly(A)⁺ RNAs derived from mouse thymus by the 3'RACE using Pfu polymerase) were treated with

- 2 3 0 -

SUPRECTM-01 (Takara, Japan) to recover cDNA, respectively.

The isolated DNAs were treated with T4 polynucleotide kinase (Wako Pure Chemical Co., Japan) to add phosphate to the end thereof and the phosphorylated DNAs 5 were ligated with pUC18 SmaI BAP (Pharmacia) by using DNA Ligation Kit (Takara, Japan) followed by transformation of E. coli JM109 to obtain 3 transformant clones, C2, C13 and C15. The clones, C13 and C15, hold the thymus-derived cDNA which is amplified by the 3'RACE and the clone, C2, holds the 10 thymus-derived cDNA which is amplified by the 3'RACE (Figure 68).

Based on the nucleotide sequences of clones, N26, N64 and N75, which are considered to hold the N-terminal region of the open reading frame (ORF) of the cDNA fragment included in p3H2-17 and the nucleotide sequences of clones, C2, C13 and 15 C15, which are considered to hold the C-terminal region of the open reading flame (ORF) of the cDNA fragment included in p3H2-17, the entire nucleotide sequence coding for the open reading flame and neighboring region of the receptor protein encoded by the cDNA included in p3H2-17 was determined. 20 To be more specific, sequencing was carried out with the primers used in the 5'RACE and 3'RACE or synthetic primers for sequencing by using a DyeDeoxy Terminator Cycle Sequencing Kit (ABI Co.), the nucleotide sequences were decoded by using a fluorescent automatic sequencer. The obtained data of the 25 DNA were analyzed by DNASIS (Hitachi System Engineering Co., Japan).

PCR errors which presumably happen to occur upon PCR have been corrected by a way of thinking that, when nucleotides between two clones which are independently produced by PCR 30 are identical (e.g. those between clones, N75 and N64, are identical) each other, the identical base is considered as correct. The determined nucleotide sequence is shown in Figure 69. The amino acid sequence is deduced based on the determined nucleotide sequence (Figure 69). Hydrophobicity 35 plotting was carried out based on the deduced amino acid sequence (Figure 70). As a result, it was learned that it was a seven transmembrane G protein coupled receptor, as

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it is suggested from the cDNA fragment included in p3H2-17.

Homology retrieval at the amino acid level indicates that it is homologous to mouse $P_{2U}^{purinoceptor}$ and chicken $P_{2Y}^{purinoceptor}$.

5 Further, the clone which are free of an error in the open reading flame (ORF) was selected and used to construct plasmids carrying the full-length ORF of the receptor protein encoded by p3H2-17. In an embodiment, the cDNA fragment held by the clone, N75, was digested with restriction enzymes, 10 DraIII and EcoRI, to obtain cDNA fragments which are the N-terminal region of the receptor protein held by p3H2-17. The C-terminal cDNA fragment encoded by C13 was digested with restriction enzymes, DraIII and EcoRI, to delete 5'-side regions from the DraIII site of the C-terminal and the long 15 fragment was obtained by the digestion of C13 with restriction enzymes, DraIII and EcoRI. The N75-derived N-terminal cDNA DraIII-EcoRI fragment was ligated with the long C13-derived DraIII-EcoRI fragment by using a DNA Ligation Kit (Takara, Japan) and transfected into Escherichia coli JM109 to obtain 20 transformant Escherichia coli JM109/pMAH2-17.

(2) Electrophysiological Measurement of Receptor Encoded by pMAH2-17

The receptor encoded by pMAH2-17 was examined electrophysiologically in Xenopus oocytes. The ORF of the 25 receptor encoded by pMAH2-17 was inserted into the XhoI-XbaI sites of pBluescriptTM II SK(+) (Stratagene) with directing the sequence thereof downstream from T7 promoter. The resulting plasmid as a template was treated with a mCAPTM mRNA Capping kit (Stratagene) to produce cRNA of this receptor 30 gene.

The cRNA was injected into Xenopus oocytes (50ng cRNA/50nl/oocyte), previously prepared according to the method disclosed in Nathan Dascal et al., Proc. Natl. Acad. Sci. USA, Vol..90, pp.6596-6600 (1993). The cRNA-injected oocytes were 35 incubated at 20 °C for 2 to 3 days and subjected to electrophysiological measurements. The measurement was carried

- 2 3 2 -

out with a microelectrode-applicable high input resistance amplifier (MEz-8300, Nippon Koden, Co., Japan), and a voltage clamping amplifier (CEz -/200, Nippon Koden, Co., Japan). The initial membrane potential of oocytes was set to -60 mV and responses (current changes of the membrane) evoked by addition of ligands were recorded with a recorder (Thermal Array recorder, Nippon Koden, Co., Japan) (Nathan Dascal et al., Proc. Natl. Acad. Sci. USA, Vol. 90, pp.6596-6600 (1993)).

Typical inward currents elicited upon activation of phospholipase C-coupled receptors were observed in oocytes injected with pMAH2-17 cRNA via stimulation by 10 μ M ATP (Figure 75). In contrast, such a current was not observed in oocytes injected with H_2O , instead of pMAH2-17 cRNA, by the ATP stimulation.

In conclusion, it is considered that the receptor encoded by pMAH2-17 cRNA is classified into a subtype within the ATP receptor, P_2 purinoceptor.

Example 20

Cloning of Rabbit Gastropyrolic Part Smooth Muscle-Derived G Protein Coupled Receptor Protein cDNA

(1) Preparation of Poly(A)⁺ RNA Fraction from Rabbit Gastropyrolic Part Smooth Muscle and Synthesis of cDNA

A total RNA was prepared from rabbit gastropyrolic part smooth muscles by the guanidine thiocyanate method (Kaplan B.B. et al., Biochem. J. 183, 181-184 (1979)) and, then, poly(A)⁺ RNA fractions were prepared with a mRNA purifying kit (Pharmacia Co.). Next, to 5 μ g of the poly(A)⁺ RNA fraction was added a random DNA hexamer (BRL Co.) as a primer, and the resulting mixture was subjected to reaction with MMLV reverse transcriptase (BRL Co.) in the buffer attached to the MMLV reverse transcriptase kit to synthesize complementary DNAs. The reaction product was extracted with phenol/chloroform (1:1), precipitated in ethanol, and was then dissolved in 30 μ l of TE.

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(2) Amplification of Receptor cDNA by PCR Using Rabbit
Gastropyrolic Part Smooth Muscle-Derived cDNA and
Sequencing

By using, as a template, 1 μ l of cDNA prepared
5 from the rabbit gastropyrolic part smooth muscle in the above
step (1), PCR amplification using the DNA primer having a
nucleotide sequence represented by SEQ ID NO: 10 and the DNA
primer having a nucleotide sequence represented by SEQ ID
NO: 4 synthesized in Example 15 was carried out.
10 A reaction solution was composed of the synthetic DNA primers
(SEQ: 5' primer sequence and 3' primer sequence) each in an
amount of 100 pM, 0.25 mM dNTPs, 1 μ l of Taq DNA polymerase
and 10 μ l of buffer attached to the enzyme kit, and the
total amount of the reaction solution was made to be 100 μ l.
15 The cycle for amplification including 96 °C for 30 sec., 45 °C
for 1 min. and 60 °C for 3 min. was repeated 25 times by using
a Thermal Cycler (Perkin-Elmer Co.). The amplified products
were confirmed relying upon 1.2% agarose gel electrophoresis
and ethidium bromide staining.

20 (3) Subcloning of PCR Product into Plasmid Vector and
Selection of Novel Receptor Candidate Clone via Decoding
Nucleotide Sequence of Inserted cDNA Region

The PCR products obtained in the above step (2)
were separated by using a 1.0% low-melting temperature agarose
25 gel, the band parts were excised from the gel with a razor
blade, and were electro-eluted, extracted with phenol and
precipitated in ethanol to recover DNAs. According to the
protocol attached to a TA Cloning Kit (Invitrogen Co.),
the recovered DNAs were subcloned to the plasmid vector,
30 pCR™ II. The recombinant vectors were introduced into
E. coli JM109 competent cells (Takara Shuzo Co., Japan) to
produce transformants. Then, transformant clones having
a cDNA-inserted fragment were selected in an LB agar
culture medium containing ampicillin, IPTG and X-gal. Only
35 transformant clones exhibiting white color were picked with
a sterilized toothstick to obtain 100 transformant clones.

- 2 3 4 -

The individual clones were cultured overnight in an LB culture medium containing ampicillin and treated with the automatic plasmid extracting machine PI-100 (Kurabo Co., Japan) to prepare plasmid DNAs. An aliquot of the DNAs thus prepared
5 was cut by EcoRI to confirm the size of the cDNA fragment that was inserted. An aliquot of the remaining DNAs was further processed with RNase, extracted with phenol/chloroform, and precipitated in ethanol so as to be condensed. Sequencing was carried out by using a DyeDeoxy terminator cycle
10 sequencing kit (ABI Co.), the DNAs were decoded by using a fluorescent automatic sequencer.

Homology retrieval was carried out based upon the determined nucleotide sequence. As a result, it was learned that a novel G protein coupled receptor protein was
15 been encoded by the cDNA fragment insert in the plasmid possessed by the transformant Escherichia coli JM109/pMN128. The nucleotide sequences of the cDNA fragments are shown in Figures 71 and 72. To further confirm this fact, by using DNASIS (Hitachi System Engineering Co., Japan) the
20 nucleotide sequences were converted into amino acid sequences [Figure 71 and Figure 72], and homology retrieval was carried out in view of hydrophobicity plotting [Figure 73] and at the amino acid sequence level to find a novel receptor protein which has 27% homology relative to hamster-derived β_2 -adrenalin receptor protein (A03159), 20% homology relative to rat-derived bradykinin receptor (type B₂) protein (A41283), 24% homology
25 relative to human-derived dopamine D₁ receptor protein (S11377) and 23% homology relative to human-derived blue sensitive opsin receptor protein (A03156). The aforementioned abbreviations in parentheses are reference numbers assigned
30 when they are registered as data to NBRF-PIR and are usually called "Accession Numbers".

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Example 21

Cloning of cDNA Comprising Whole Coding Regions for Receptor Protein from Human-Derived DNA Library

The DNA library constructed by Clontech wherein
5 λ gt11 phage vector is used (CLONTECH Laboratories, Inc.; CLH L1008b) was employed as a human placenta-derived cDNA library. The human placenta cDNA library (1×10^5 pfu (plaque forming units)) was thermally denatured. By using the human placenta-derived cDNA library, PCR amplification using
10 the DNA primer having a nucleotide sequence represented by SEQ ID NO: 20 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 23 synthesized in Example 19 was carried out.

(Nucleotide sequence of synthesized primer)

15 ① 5'-TAGTGTGTGGAGTCGTGTGGCTGGCTG-3'

(SEQ ID NO: 20)

② 5'-ACAGGACCTGCTGGGCCATCCTGGCGACACA-3'

(SEQ ID NO: 23)

20 The isolated DNA was subcloned using a TA Cloning Kit (Invitrogen Co.) and sequencing was carried out. Figure 76 shows a nucleotide sequence of obtained cDNA fragment, ph3H2-17. As a result, it was learned that ph3H2-17 is highly homologous to the mouse purinoceptor cDNA fragment, p3H2-17. It is strongly suggested that the human-derived cDNA fragment is a
25 partial nucleotide sequence of human purinoceptor.

Based on the nucleotide sequence of ph3H2-17 which was sequenced, the following 2 primers were synthesized:

(Nucleotide sequence of synthesized primer)

30 ③ 5'-ACAGCCATCTTCGCTGCCACAGGCAT-3'

(SEQ ID NO: 58)

④ 5'-AGACAGTAGCAGGCCAGCAGGGCAGCAA-3'

(SEQ ID NO: 59)

- 2 3 6 -

The above synthetic 2 primers were each used in combination with λ gt 11 primers (Takara, Japan; catalogue 3864) for obtaining full-length human purinoceptor cDNA. Thus, using thermally denatured, human placenta-derived λ gt 11 cDNA libraries (CLONTECH; CLHL 1008b), first RCR amplification using a combination of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 20 with λ gt 11 Forward primer, of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 20 with λ gt 11 Reverse primer, of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 23 with λ gt 11 Forward primer, and of the DNA primer having a nucleotide sequence represented by SEQ ID NO: 23 with λ gt 11 Reverse primer was carried out with Ex Tag polymerase (Takara, Japan) (30 cycles; 95°C/30 seconds, 55 °C/60 seconds, and 72 °C/60 seconds), respectively.

Next, by using a 1/50 of the 1st PCR product, second RCR amplification was carried in the same manner as in the first PCR except for using the DNA primer having a nucleotide sequence represented by SEQ ID NO: 58 in place of SEQ ID NO: 20 and the DNA primer having a nucleotide sequence represented by SEQ ID NO: 59 in place of SEQ ID NO: 23 (30 cycles; 95°C/30 seconds, 65 °C/60 seconds and 72 °C/60 seconds). The amplified product DNA was subcloned using a TA Cloning Kit (Invitrogen Co.) and sequencing was carried out for three clones each of 5' and 3' sides (Figure 77).

Based on the amino acid sequence (Figure 77) deduced from the determined nucleotide sequence of human purinoceptor cDNA as shown in Figure 77, hydrophobicity plotting was carried out (Figure 78). As a result, it was learned that the human-derived receptor is a novel seven transmembrane G protein coupled receptor, similarly to the mouse type. It was also learned that the deduced amino acid sequence of human receptor has 87% homology relative to the amino acid sequence of mouse purinoceptor and its amino acid residues are well conserved (Figure 79).

Clones free of PCR errors which often occur in a PCR amplification were selected and restriction enzyme regions

- 2 3 7 -

comprising overlapping areas were obtained therefrom.

The restriction enzyme regions thus obtained were subjected to construction of plasmid phAH2-17 having a full-length open reading frame of human purinoceptor cDNA. The plasmid phAH2-17
5 is possessed by transformant Escherichia coli JM109/phAH2-17.

The DNA primers of the present invention allow efficient amplification of DNAs that encode G protein coupled receptor proteins. This makes it possible to efficiently screen for the DNAs coding for G protein coupled receptor
10 proteins and to accomplish the cloning.

The G protein coupled receptor protein of the present invention and their G protein coupled receptor protein-encoding DNA are advantageously useful in:

- ① determining ligands,
- 15 ② obtaining antibodies and an antisera,
- ③ constructing systems for expressing recombinant receptor proteins,
- ④ investigating or developing receptor-binding assay systems and screening for pharmaceutical candidate compounds, by using
20 the above expression system
- ⑤ designing drugs based upon comparisons with ligands and receptors having a structure similar or analogous thereto,
- ⑥ preparing probes and/or PCR primers in gene diagnosis, and
- ⑦ gene manipulating therapy.

25 In particular, discovering the structure and properties of the G protein coupled receptor will lead to the development of unique pharmaceuticals acting upon these systems.

The practice of the present invention will employ, otherwise indicated, conventional techniques of molecular
30 biology, microbiology, recombinant DNA, pharmacology, immunology, bioscience, and medical technology, which are within the skill of the art. All patents, patent applications, and publications mentioned herein, both supra and infra, are hereby incorporated herein by reference.

- 2 3 8 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Takeda Chemical Industries, Ltd.
- (B) STREET: 1-1, Doshomachi 4-chome, Chuo-ku
- (C) CITY: Osaka-shi
- (D) STATE: Osaka
- (E) COUNTRY: Japan
- (F) POSTAL CODE (ZIP): 541

(ii) TITLE OF INVENTION: G Protein Coupled Receptor Protein,
Production, And Use Thereof

(iii) NUMBER OF SEQUENCES: 61

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CGTGGSCMTS STGGGCAACN YCCTG 25

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is A, G, C, or T

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTNGWRRGGC ANCCAGCAGA KGGCAA 27

- 2 3 9 -

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA
- (iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTCGCGCYM TNRGYATGGA YCGNTAT 27

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA
- (iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CATGTRGWAG GGAANCCAGS AMANRARAA 30

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA
- (iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CTGACYGYTC TNRSNRYTGA CMGVTAC 27

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Single

- 2 4 0 -

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

CTGACYGYTC TNRSNRYTGA CMGVTAT 27

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CTCGCSCYTM TNRGYATGGA YCGNTAC 27

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GATGTGRTAR GGSRNCCAAC AGANGRYAAA 30

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

- 2 4 1 -

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GATGTGRTAR GGSRNCCAAC AGANGRYGAA 30

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GYCACCAACN WSTTCATCCT SWNHCTG 27

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ASNSANRAAG SARTAGANGA NRGGRTT 27

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TGNTSSTKMT NGSNGTKGTN GGNAA 25

- 2 4 2 -

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

AYCKGTAYCK GTCCANKGWN ATKGC 25

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CATKKCCSTG GASAGNTAYN TRGC 24

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GWWGGGSACK CAGCASANGG CRAA 24

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18

- 2 4 3 -

(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: 15th N is A, G, C, or T
6th, 9th, 10th & 12th Ns are inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

ARYYTNGCNN TNGCNGAY 18

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: 13th, 15th, 16th & 18th Ns are
each A, G, C, or T
1st, 4th, 6th Ns are inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

NGGNANCCAR CANANNRNRNRA A 21

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GCCTSNTNRN SATGWSTGTG GANMGNT 27

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single

- 2 4 4 -

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GAWSNTGMYN ANRTGGWAGG GNANCCA 27

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TAGTGTGTGG AGTCGTGTGG CTGGCTG 27

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

AGTCTTTGCT GCCACAGGCA TCCAGCG 27

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CAAGCCAGTA AGGCTATGAA GGGCAGCAAG 30

- 2 4 5 -

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

ACAGGACCTG CTGGGCCATC CTGGCGACAC A 31

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 91
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Leu Val Leu Val Ile Ala Arg Val Arg Arg Leu His Asn Val Thr Asn
1 5 10 15

Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu Met Cys Thr Ala
20 25 30

Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly Trp Val
35 40 45

Phe Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Pro Val Thr
50 55 60

Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr
65 70 75 80

Val Val Leu Val His Pro Leu Arg Arg Arg Ile
85 90

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Gly Leu Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val Ile Leu Leu

- 2 4 6 -

1	5	10	15
Ser	Tyr	Val	Arg
20		Val	Val
Ser	Val	Lys	Leu
		Arg	Arg
		Asn	Arg
		Arg	Val
		Val	Pro
			Gly
35		25	30
Cys	Val	Thr	Gln
		Ser	Gln
		Ala	Asp
		Trp	Asp
		Arg	Ala
		Arg	Arg
		Arg	Arg
		Arg	Arg
Thr	Phe	Cys	Leu
		Leu	Val
		Val	Val
		Val	Val
		Val	Val
50		55	

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 370
- (B) TYPE: Amino acid
- (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Met	Ala	Ser	Ser	Thr	Thr	Arg	Gly	Pro	Arg	Val	Ser	Asp	Leu	Phe	Ser
1						5				10					15
Gly	Leu	Pro	Pro	Ala	Val	Thr	Thr	Pro	Ala	Asn	Gln	Ser	Ala	Glu	Ala
						20			25					30	
Ser	Ala	Gly	Asn	Gly	Ser	Val	Ala	Gly	Ala	Asp	Ala	Pro	Ala	Val	Thr
						35			40					45	
Pro	Phe	Gln	Ser	Leu	Gln	Leu	Val	His	Gln	Leu	Lys	Gly	Leu	Ile	Val
						50			55					60	
Leu	Leu	Tyr	Ser	Val	Val	Val	Val	Gly	Leu	Val	Gly	Asn	Cys	Leu	
						65			70		75			80	
Leu	Val	Leu	Val	Ile	Ala	Arg	Val	Arg	Arg	Leu	His	Asn	Val	Thr	Asn
						85			90					95	
Phe	Leu	Ile	Gly	Asn	Leu	Ala	Leu	Ser	Asp	Val	Leu	Met	Cys	Thr	Ala
						100			105					110	
Cys	Val	Pro	Leu	Thr	Leu	Ala	Tyr	Ala	Phe	Glu	Pro	Arg	Gly	Trp	Val
						115			120					125	
Phe	Gly	Gly	Gly	Leu	Cys	His	Leu	Val	Phe	Phe	Leu	Gln	Pro	Val	Thr
						130			135					140	
Val	Tyr	Val	Ser	Val	Phe	Thr	Leu	Thr	Thr	Ile	Ala	Val	Asp	Arg	Tyr
						145			150		155			160	
Val	Val	Leu	Val	His	Pro	Leu	Arg	Arg	Ile	Ser	Leu	Arg	Leu	Ser	
						165			170					175	
Ala	Tyr	Ala	Val	Leu	Ala	Ile	Trp	Ala	Leu	Ser	Ala	Val	Leu	Ala	Leu

- 2 4 7 -

180	185	190
Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val		
195	200	205
Arg Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Leu		
210	215	220
Tyr Ala Trp Gly Leu Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val		
225	230	235
Ile Leu Leu Ser Tyr Val Arg Val Ser Val Lys Leu Arg Asn Arg Val		
245	250	255
Val Pro Gly Cys Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg		
260	265	270
Arg Arg Arg Thr Phe Cys Leu Leu Val Val Val Val Val Phe Ala		
275	280	285
Val Cys Trp Leu Pro Leu His Val Phe Asn Leu Leu Arg Asp Leu Asp		
290	295	300
Pro His Ala Ile Asp Pro Tyr Ala Phe Gly Leu Val Gln Leu Leu Cys		
305	310	315
His Trp Leu Ala Met Ser Ser Ala Cys Tyr Asn Pro Phe Ile Tyr Ala		
325	330	335
Trp Leu His Asp Ser Phe Arg Glu Glu Leu Arg Lys Leu Leu Val Ala		
340	345	350
Trp Pro Arg Lys Ile Ala Pro His Gly Gln Asn Met Thr Val Ser Val		
355	360	365
Val Ile		
370		

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Leu Val Leu Val Ile Ala Arg Val Arg Arg Leu Tyr Asn Val Thr Asn
1 5 10 15

Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu Met Cys Thr Ala
20 25 30

Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly Trp Val

- 2 4 8 -

35	40	45
Phe Gly Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Ala Val Thr		
50	55	60
Val Tyr Val Ser Val Phe Thr Leu Thr Thr Ile Ala Val Asp Arg Tyr		
65	70	75
80		
Val Val Leu Val His Pro Leu Arg Arg Arg Ile Ser Leu Arg Leu Ser		
85	90	95
Ala Tyr Ala Val Leu Ala Ile Trp Val Leu Ser Ala Val Leu Ala Leu		
100	105	110
Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val		
115	120	125
Arg Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Leu		
130	135	140
Tyr Ala Trp Gly Leu Leu Val Thr Tyr Leu Leu Pro Leu Leu Val		
145	150	155
160		
Ile Leu Leu Ser Tyr Ala Arg Val Ser Val Lys Leu Arg Asn Arg Val		
165	170	175
Val Pro Gly Arg Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg		
180	185	190
Arg Arg Arg Thr Phe Cys Leu Leu Val Val Val Val Val Val		
195	200	205

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 126
- (B) TYPE: Amino acid
- (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Val Val Leu Val His Pro Leu Arg Arg Arg Ile Ser Leu Arg Leu Ser		
1	5	10
15		
Ala Tyr Ala Val Leu Gly Ile Trp Ala Leu Ser Ala Val Leu Ala Leu		
20	25	30
Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro His Asp Val		
35	40	45
Ser Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln Ile		
50	55	60
Tyr Ala Trp Gly Leu Leu Leu Gly Thr Tyr Leu Leu Pro Leu Leu Ala		

- 2 4 9 -

65	70	75	80
Ile Leu Leu Ser Tyr Val Arg Val Ser Val Lys Leu Arg Asn Arg Val			
85	90	95	
Val Pro Gly Ser Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg			
100	105	110	
Arg Arg Arg Thr Phe Cys Leu Leu Val Val Val Val Val Val			
115	120	125	

(2) INFORMATION FOR SEQ ID NO: 29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 273
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE
 - (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

```

CTGGTGCTGG TGATCGCGCG GGTGCGCCGG CTGCACAAACG TGACGAACCTT CCTCATCGGC 60
AACCTGGCCT TGTCCGACGT GCTCATGTGC ACCGCCTGCG TGCCGCTCAC GCTGGCCTAT 120
GCCTTCGAGC CACGCCGCTG GGTGTTCGGC GGCGGCCTGT GCCACCTGGT CTTCTTCCTG 180
CAGCCGGTCA CCGTCTATGT GTCCGGTGTTC ACGCTCACCA CCATCGCAGT GGACCGGTAC 240
GTCGTGCTGG TGCACCCGCT GAGGCGGCGC ATC

```

(2) INFORMATION FOR SEQ ID NO: 30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 177
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE
 - (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

```

GGCCTGCTGC TGGTCACCTA CCTGCTCCCT CTGCTGGTCA TCCTCCTGTC TTACGTCCGG 60
GTGTCAGTGA AGCTCCGCAA CCGCGTGGTG CCGGGCTGCG TGACCCAGAG CCAGGCCGAC 120
TGGGACCGCG CTCGGCGCCG GCGCACCTTC TGCTTGCTGG TGGTGGTCGT GGTGGTG

```

- 2 5 0 -

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1110
 (B) TYPE: Nucleic acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
 (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ATGGCCTCAT CGACCACTCG GGGCCCCAGG GTTTCTGACT TATTTTCTGG GCTGCCGCCG	60
GGGGTCACAA CTCCCGCCAA CCAGAGCGCA GAGGCCTCGG CGGGCAACGG GTCGGTGGCT	120
GGCGCGGACG CTCCAGCCGT CACGCCCTTC CAGAGCCTGC AGCTGGTGCA TCAGCTGAAG	180
GGGCTGATCG TGCTGCTCTA CAGCGTCGTG GTGGTCGTGG GGCTGGTGGG CAACTGCCTG	240
CTGGTGCTGG TGATCGCGCG GGTGCGCCGG CTGCACAACG TGACGAACCTT CCTCATCGC	300
AACCTGGCCT TGTCCGACGT GCTCATGTGC ACCGCCTGCG TGCCGCTCAC GCTGGCCTAT	360
GCCTTCGAGC CACGCGGCTG GGTGTTCGC GGCAGGCTGT GCCACCTGGT CTTCTTCCTG	420
CAGCCGGTCA CCGTCTATGT GTCGGTGTTC ACGCTCACCA CCATCGCAGT GGACCGCTAC	480
GTCGTGCTGG TGCACCCCGCT GAGGCGGCCGC ATCTCGCTGC GCCTCAGCGC CTACGCTGTG	540
CTGGCCATCT GGGCGCTGTC CGCGGTGCTG GCGCTGCCCG CCGCCGTGCA CACCTATCAC	600
GTGGAGCTCA AGCCGCACGA CGTGCGCCTC TGCGAGGAGT TCTGGGGCTC CCAGGAGCGC	660
CAGCGCCAGC TCTACGCCTG GGGGCTGCTG CTGGTCACCT ACCTGCTCCC TCTGCTGGTC	720
ATCCTCCTGT CTTACGTCCG GGTGTCAGTG AAGCTCCGCA ACCGCGTGGT GCCAGGCTG	780
GTGACCCAGA GCCAGGCCGA CTGGGACCGC GCTCGGCAGCC GGCGCACCTT CTGCTTGCTG	840
GTGGTGGTCG TGGTGGTGTG CGCCGTCTGC TGGCTGCCGC TGCACGTCTT CAACCTGCTG	900
CGGGACCTCG ACCCCCCACGC CATCGACCCCT TACGCCCTTG GGCTGGTGCA GCTGCTCTGC	960
CACTGGCTCG CCATGAGTTC GGCCTGCTAC AACCCCTTC TCTACGCCTG GCTGCACGAC	1020
AGCTTCCGCG AGGAGCTGCG CAAACTGTTG GTCGCTTGGC CCCGCAAGAT AGCCCCCCCAT	1080
GGCCAGAATA TGACCGTCAG CGTGGTCATC	1110

(2) INFORMATION FOR SEQ ID NO: 32:

- 2 5 1 -

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 618
 (B) TYPE: Nucleic acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
 (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CTGGTGTGG	TGATCGCGCG	GGTGCGCCGG	CTGTACAACG	TGACGAATTT	CCTCATCGGC	60
AACCTGGCCT	TGTCCGACGT	GCTCATGTGC	ACCGCCTGCG	TGCCGCTCAC	GCTGGCCTAT	120
GCCTTCGAGC	CACGCCGCTG	GGTGTTCGGC	GGCGGCCCTGT	GCCACCTGGT	CTTCTTCCTG	180
CAGGCGGTCA	CCGTCTATGT	GTCGGTGTTC	ACGCTCACCA	CCATCGCAGT	GGACCGCTAC	240
GTCTGTGG	TGCACCCGCT	GAGGCGGCCG	ATCTCGCTGC	GCCTCAGCGC	CTACGCTGTG	300
CTGGCCATCT	GGGTGCTGTC	CGCGGTGCTG	GCGCTGCCCG	CCGCCGTGCA	CACCTATCAC	360
GTGGAGCTCA	AGCCGCACGA	CGTGCGCCTC	TGCGAGGAGT	TCTGGGGCTC	CCAGGAGCGC	420
CAGCGCCAGC	TCTACGCCTG	GGGGCTGCTG	CTGGTCACCT	ACCTGCTCCC	TCTGCTGGTC	480
ATCCTCCTGT	CTTACGCCCG	GGTGTCACTG	AAGCTCCGCA	ACCGCGTGGT	GCCGGGCCGC	540
GTGACCCAGA	GCCAGGCCGA	CTGGGACCGC	GCTCGGCGCC	GGCGCACCTT	CTGCTTGCTG	600
GTGGTGGTCG	TGGTGGTG					618

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 378
 (B) TYPE: Nucleic acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
 (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GTGGTTCTGG	TGCACCCGCT	ACGTCGGCGC	ATTTCACTGA	GGCTCAGCGC	CTACGCGGTG	60
CTGGGCATCT	GGGCTCTATC	TGCAGTGCTG	GCGCTGCCCG	CCGCCGTGCA	CACCTACCAT	120
GTGGAGCTCA	AGCCCCACGA	CGTGAGCCTC	TGCGAGGAGT	TCTGGGGCTC	GCAGGAGCGC	180

- 2 5 2 -

CAACGCCAGA TCTACGCCTG GGGGCTGCTT CTGGGCACCT ATTTGCTCCC CCTGCTGGCC	240
ATCCTCCTGT CTTACGTACG GGTGTCAGTG AAGCTGAGGA ACCGCGTGGT GCCTGGCAGC	300
GTGACCCAGA GTCAAGCTGA CTGGGACCGA GCGCGTCGCC GCCGCACTTT CTGTCTGCTG	360
GTGGTGGTGG TGGTAGTG	378

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 70
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Val Cys His Val Ile Phe Lys Asn Gln Arg Met His Ser Ala Thr Ser			
1	5	10	15
Leu Phe Ile Val Asn Leu Ala Val Ala Asp Ile Met Ile Thr Leu Ile			
20	25	30	
Asn Thr Pro Phe Thr Leu Val Arg Phe Val Asn Ser Thr Trp Ile Phe			
35	40	45	
Gly Lys Gly Met Cys His Val Ser Arg Phe Ala Gln Tyr Cys Ser Leu			
50	55	60	
His Val Ser Ala Leu Thr			
65	70		

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 71
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Glu Pro Ala Asp Leu Phe Trp Lys Asn Leu Asp Leu Pro Thr Phe Ile			
1	5	10	15
Leu Leu Asn Ile Leu Pro Leu Leu Ile Ile Ser Val Ala Tyr Val Arg			
20	25	30	
Val Thr Lys Lys Leu Trp Leu Cys Asn Met Ile Val Asp Val Thr Thr			
35	40	45	
Glu Gln Tyr Phe Ala Leu Arg Pro Lys Lys Lys Lys Thr Ile Lys Met			
50	55	60	

- 2 5 3 -

Leu Met Leu Val Val Val Leu
65 70

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 210
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

GTCTGTCATG TCATCTCAA GAACCAGCGA ATGCACTCGG CCACCAGCCT CTTCATCGTC	60
AACCTGGCAG TTGCCGACAT AATGATCACG CTGCTAACAA CCCCCTTCAC TTTGGTTCGC	120
TTTGTGAACA GCACATGGAT ATTGGGAAG GGCATGTGCC ATGTCAGCCG CTTTGCCCAG	180
TACTGCTCAC TGCACGTCTC AGCACTGACA	210

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 213
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

GAGCCAGCTG ACCTCTCTG GAAGAACCTG GACTTGCCCA CCTTCATCCT GCTAACATC	60
CTGCCCCCTCC TCATCATCTC TGTGGCCTAC GTTCGTGTGA CCAAGAACT GTGGCTGTGT	120
AATATGATTG TCGATGTGAC CACAGAGCAG TACTTTGCCG TGCGGCCAA AAAGAAGAAG	180
ACCATCAAGA TGTTGATGCT GGTGGTAGTC CTC	213

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

- 2 5 4 -

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Ala Ser Trp His Lys Arg Gly Gly Arg Arg Ala Ala Trp Val Val Cys
1 5 10 15

Gly Val Val Trp Leu Ala Val Thr Ala Gln Cys Leu Pro Thr Ala Val
20 25 30

Phe Ala Ala Thr Gly Ile Gln Arg Asn Arg Thr Val Cys Tyr Asp Leu
35 40 45

Ser Pro Pro Ile Leu Ser Thr Arg Tyr Leu Pro Tyr Gly Met Ala Leu
50 55 60

Thr Val Ile Gly Phe Leu Leu Pro Phe Ile Ala Leu Leu Ala Cys Tyr
65 70 75 80

Cys Arg Met Ala Arg Arg Leu Cys Arg Gln Asp Gly Pro Ala Gly Pro
85 90 95

Val Ala Gln Glu Arg Arg Ser Lys Ala Ala Arg Met Ala Val Val Val
100 105 110

Ala Ala Val
115

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 328
- (B) TYPE: Amino acid
- (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Met Glu Gln Asp Asn Gly Thr Ile Gln Ala Pro Gly Leu Pro Pro Thr
1 5 10 15

Thr Cys Val Tyr Arg Glu Asp Phe Lys Arg Leu Leu Leu Thr Pro Val
20 25 30

Tyr Ser Val Val Leu Val Val Gly Leu Pro Leu Asn Ile Cys Val Ile
35 40 45

Ala Gln Ile Cys Ala Ser Arg Arg Thr Leu Thr Arg Ser Ala Val Tyr
50 55 60

Thr Leu Asn Leu Ala Leu Ala Asp Leu Met Tyr Ala Cys Ser Leu Pro
65 70 75 80

- 2 5 5 -

Leu	Leu	Ile	Tyr	Asn	Tyr	Ala	Arg	Gly	Asp	His	Trp	Pro	Phe	Gly	Asp
				85					90						95
Leu	Ala	Cys	Arg	Phe	Val	Arg	Phe	Leu	Phe	Tyr	Ala	Asn	Leu	His	Gly
				100				105						110	
Ser	Ile	Leu	Phe	Leu	Thr	Cys	Ile	Ser	Phe	Gln	Arg	Tyr	Leu	Gly	Ile
					115			120						125	
Cys	His	Pro	Leu	Ala	Ser	Trp	His	Lys	Arg	Gly	Gly	Arg	Arg	Ala	Ala
					130			135					140		
Trp	Val	Val	Cys	Gly	Val	Val	Trp	Leu	Ala	Val	Thr	Ala	Gln	Cys	Leu
					145			150			155			160	
Pro	Thr	Ala	Val	Phe	Ala	Ala	Thr	Gly	Ile	Gln	Arg	Asn	Arg	Thr	Val
					165			170					175		
Cys	Tyr	Asp	Leu	Ser	Pro	Pro	Ile	Leu	Ser	Thr	Arg	Tyr	Leu	Pro	Tyr
					180			185					190		
Gly	Met	Ala	Leu	Thr	Val	Ile	Gly	Phe	Leu	Leu	Pro	Phe	Ile	Ala	Leu
					195			200					205		
Leu	Ala	Cys	Tyr	Cys	Arg	Met	Ala	Arg	Arg	Leu	Cys	Arg	Gln	Asp	Gly
					210			215					220		
Pro	Ala	Gly	Pro	Val	Ala	Gln	Glu	Arg	Arg	Ser	Lys	Ala	Ala	Arg	Met
					225			230			235			240	
Ala	Val	Val	Val	Ala	Ala	Val	Phe	Ala	Ile	Ser	Phe	Leu	Pro	Phe	His
					245			250					255		
Ile	Thr	Lys	Thr	Ala	Tyr	Leu	Ala	Val	Arg	Ser	Thr	Pro	Gly	Val	Ser
					260			265					270		
Cys	Pro	Val	Leu	Glu	Thr	Phe	Ala	Ala	Ala	Tyr	Lys	Gly	Thr	Arg	Pro
					275			280					285		
Phe	Ala	Ser	Val	Asn	Ser	Val	Leu	Asp	Pro	Ile	Leu	Phe	Tyr	Phe	Thr
					290			295					300		
Gln	Gln	Lys	Phe	Arg	Arg	Gln	Pro	His	Asp	Leu	Leu	Gln	Arg	Leu	Thr
					305			310			315			320	
Ala	Lys	Trp	Gln	Arg	Gln	Arg	Val								
					325										

(2) INFORMATION FOR SEQ ID NO: 40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 345
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear

- 2 5 6 -

(ii) MOLECULE TYPE: CDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

GCTTCCTGGC ACAAGCGTGG AGGTGCCGT GCTGCTTGGG TAGTGTGTGG AGTCGTGTGG	60
CTGGCTGTGA CAGCCCAGTG CCTGCCACG GCAGTCTTG CTGCCACAGG CATCCAGCGC	120
AACCGCACTG TGTGCTACGA CCTGAGCCA CCCATCCTGT CTACTCGCTA CCTGCCCTAT	180
GGTATGGCCC TCACGGTCAT CGGCTTCTTG CTGCCCTCA TAGCCTTACT GGCTTGTAT	240
TGTCGCATGG CCCGCCGCCT GTGTGCCAG GATGGCCAG CAGGTCTGT GGCCCAAGAG	300
CGGCGCAGCA AGGCGGCTCG TATGGCTGTG GTGGTGGCAG CTGTC	345

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 984
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: CDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

ATGGAGCAGG ACAATGGCAC CATCCAGGCT CCAGGCTTGC CGCCCACAC CTGCGTCTAC	60
CGTGAGGATT TCAAGCGACT GCTGCTAACCC CGGGTATACT CGGTGGTGCT GGTGGTCGGC	120
CTGCCACTGA ACATCTGCGT CATTGCCAG ATCTGCGCAT CCCGCCGGAC CCTGACCCGT	180
TCCGCTGTGT ACACCCTGAA CCTGGCACTG GCGGACCTGA TGTATGCCCTG TTCACTACCC	240
CTACTTATCT ATAACATACGC CAGAGGGGAC CACTGGCCCT TCGGAGACCT CGCCTGCCGC	300
TTTGTACGCT TCCTCTTCTA TGCCAATCTA CATGGCAGCA TCCTGTTCCCT CACCTGCATT	360
AGCTTCCAGC GCTACCTGGG CATCTGCCAC CCCCTGGCTT CCTGGCACAA GCGTGGAGGT	420
CGCCGTGCTG CTTGGGTAGT GTGTGGAGTC GTGTGGCTGG CTGTGACAGC CCAGTGCCTG	480
CCCACGGCAG TCTTGCTGC CACAGGCATC CAGCGCAACC GCACTGTGTG CTACGACCTG	540
AGCCCCACCCA TCCTGTCTAC TCGCTACCTG CCCTATGGTA TGGCCCTCAC GGTCATCGGC	600
TTCTTGCTGC CCTTCATAGC CTTACTGGCT TGTTATTGTC GCATGGCCCG CCGCCTGTGT	660

- 2 5 7 -

CGCCAGGATG GCCCAGCAGG TCCTGTGGCC CAAGAGCGGC GCAGCAAGGC GGCTCGTATG	720
GCTGTGGTGG TGGCAGCTGT CTTGCCATC AGCTTCCTGC CTTTCCACAT CACCAAGACA	780
GCCTACTTGG CTGTGCCGTC CACGCCCGGT GTCTCTGCC CTGTGCTGGA GACCTTCGCT	840
GCTGCCTACA AAGGCACTCG GCCCTTCGCC AGTGTCAACA GTGTTCTGGA CCCCCATTCTC	900
TTCTACTTCA CACAACAGAA GTTCCGGCGG CAACCCCACG ATCTCTTACA GAGGGCTCACA	960
GCCAAGTGGC AGAGGCAGAG AGTC	984

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128
- (B) TYPE: Amino acid
- (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

Ala Ala Met Ser Val Asp Arg Tyr Val Ala Ile Val His Ser Arg Arg	
1 5 10 15	
Ser Ser Ser Leu Arg Val Ser Arg Asn Ala Leu Leu Gly Val Gly Phe	
20 25 30	
Ile Trp Ala Leu Ser Ile Ala Met Ala Ser Pro Val Ala Tyr His Gln	
35 40 45	
Arg Leu Phe His Arg Asp Ser Asn Gln Thr Phe Cys Trp Glu Gln Trp	
50 55 60	
Pro Asn Lys Leu His Lys Lys Ala Tyr Val Val Cys Thr Phe Val Phe	
65 70 75 80	
Gly Tyr Leu Leu Pro Leu Leu Ile Cys Phe Cys Tyr Ala Lys Val	
85 90 95	
Leu Asn His Leu His Lys Lys Leu Lys Asn Met Ser Lys Lys Ser Glu	
100 105 110	
Ala Ser Lys Lys Thr Ala Gln Thr Val Leu Val Val Val Val Val	
115 120 125	

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 384
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

- 2 5 8 -

(ix) FEATURE
 (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GCCGGCGATGT CTGTGGATCG CTACGTGGCC ATTGTGCACT CGCGGGCGTC	CTCCTCCCTC	60
AGGGTGTCCC GCAACGCACT GCTGGCGTG GGCTTCATCT GGGCGCTGTC	CATGCCATG	120
GCCTCGCCGG TGGCCTACCA CCAGCGTCTT TTCCATCGGG ACAGCAACCA	GACCTTCTGC	180
TGGGAGCAGT GGCCCAACAA GCTCCACAAG AAGGCTTACG TGGTGTGCAC	TTTCGTCTTT	240
GGGTACCTTC TGCCCTTAECT GCTCATCTGC TTTTGCTATG CCAAGGTCT	TAATCATCTG	300
CATAAAAAGC TGAAAAAACAT GTCAAAAAAG TCTGAAGCAT CCAAGAAAAA	GACTGCACAG	360
ACCGTCCTGG TGGTCGTTGT AGTA		384

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 71
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Val Leu Trp Phe Phe Gly Phe Ser Ile Lys Arg Thr Pro Phe Ser Val			
1	5	10	15

Tyr Phe Leu His Leu Ala Ser Ala Asp Gly Ala Tyr Leu Phe Ser Lys		
20	25	30

Ala Val Phe Ser Leu Leu Asn Ala Gly Gly Phe Leu Gly Thr Phe Ala		
35	40	45

His Tyr Val Arg Ser Val Ala Arg Val Leu Gly Leu Cys Ala Phe Val		
50	55	60

Ala Gly Val Ser Leu Leu Pro		
65	70	

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 215
 (B) TYPE: Nucleic acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

- 2 5 9 -

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GTGCTCTGGT TCTTCGGCTT CTCCATCAAG AGGACCCCT TCTCCGTCTA CTTCCCTGCAC	60
CTGGCCAGCG CCGACGGCGC CTACCTCTTC AGCAAGGCCG TGTTCTCCCT GCTGAACGCC	120
GGCGGCTTCC TGGGCACCTT CGCCCACATAT GTGCGCAGCG TGGCCCGGGT GCTGGGGCTC	180
TGCGCCTTCG TGGCGGGCGT GAGCCTCCTG CCGGC	215

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 348
 (B) TYPE: Amino acid
 (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Met Glu Leu Ala Met Val Asn Leu Ser Glu Gly Asn Gly Ser Asp Pro			
1	5	10	15

Glu Pro Pro Ala Pro Glu Ser Arg Pro Leu Phe Gly Ile Gly Val Glu		
20	25	30

Asn Phe Ile Thr Leu Val Val Phe Gly Leu Ile Phe Ala Met Gly Val		
35	40	45

Leu Gly Asn Ser Leu Val Ile Thr Val Leu Ala Arg Ser Lys Pro Gly		
50	55	60

Lys Pro Arg Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu Ser Ile Ala			
65	70	75	80

Asp Leu Ala Tyr Leu Leu Phe Cys Ile Pro Phe Gln Ala Thr Val Tyr		
85	90	95

Ala Leu Pro Thr Trp Val Leu Gly Ala Phe Ile Cys Lys Phe Ile His		
100	105	110

Tyr Phe Phe Thr Val Ser Met Leu Val Ser Ile Phe Thr Leu Ala Ala		
115	120	125

Met ser Val Asp Arg Tyr Val Ala Ile Val His Ser Arg Arg Ser Ser		
130	135	140

Ser Leu Arg Val Ser Arg Asn Ala Leu Leu Gly Val Gly Phe Ile Trp			
145	150	155	160

Ala Leu Ser Ile Ala Met Ala Ser Pro Val Ala Tyr His Gln Arg Leu		
165	170	175

- 2 6 0 -

Phe His Arg Asp Ser Asn Gln Thr Phe Cys Trp Glu Gln Trp Pro Asn		
180	185	190
Lys Leu His Lys Lys Ala Tyr Val Val Cys Thr Phe Val Phe Gly Tyr		
195	200	205
Leu Leu Pro Leu Leu Ile Cys Phe Cys Tyr Ala Lys Val Leu Asn		
210	215	220
His Leu His Lys Lys Leu Lys Asn Met Ser Lys Lys Ser Glu Ala Ser		
225	230	235
240		
Lys Lys Lys Thr Ala Gln Thr Val Leu Val Val Val Val Phe Gly		
245	250	255
Ile Ser Trp Leu Pro His His Val Val His Leu Trp Ala Glu Phe Gly		
260	265	270
Ala Phe Pro Leu Thr Pro Ala Ser Phe Phe Phe Arg Ile Thr Ala His		
275	280	285
Cys Leu Ala Tyr Ser Asn Ser Ser Val Asn Pro Ile Ile Tyr Ala Phe		
290	295	300
Leu Ser Glu Asn Phe Arg Lys Ala Tyr Lys Gln Val Phe Lys Cys His		
305	310	315
320		
Val Cys Asp Glu Ser Pro Arg Ser Glu Thr Lys Glu Asn Lys Ser Arg		
325	330	335
Met Asp Thr Pro Pro Ser Thr Asn Cys Thr His Val		
340	345	

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1044
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE
(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

ATGGAACCTGG CTATGGTGAA CCTCAGTGAA GGGAAATGGGA GCGACCCAGA GCCGCCAGCC	60
CCGGAGTCCA GGCGCTCTT CGGCATTGGC GTGGAGAACT TCATTACGCT GGTAGTGT	120
GGCCTGATTT TCGCGATGGG CGTGCTGGGC AACAGCCTGG TGATCACCGT GCTGGCGCGC	180
AGCAAACCAG GCAACCCCCCG CAGCACCAACC AACCTGTTTA TCCTCAATCT GAGCATCGCA	240

- 2 6 1 -

GACCTGGCCT	ACCTGCTCTT	CTGCATCCCT	TTTCAGGCCA	CCGTGTATGC	ACTGCCACC	300
TGGGTGCTGG	GCGCCTTCAT	CTGCAAGTTT	ATACACTACT	TCTTCACCGT	GTCCATGCTG	360
GTGAGCATCT	TCACCCCTGGC	CGCGATGTCT	GTGGATCGCT	ACGTGGCCAT	TGTGCACTCG	420
CGGCGCTCCT	CCTCCCTCAG	GGTGTCCCAG	AACGCACACTGC	TGGGCGTGGG	CTTCATCTGG	480
GCGCTGTCCA	TCGCCATGGC	CTCGCCGGTG	GCCTACCACC	AGCGTCTTTT	CCATCGGGAC	540
AGCAACCAGA	CCTTCTGCTG	GGAGCAGTGG	CCCAACAAGC	TCCACAAGAA	GGCTTACGTG	600
GTGTGCACTT	TCGTCTTG	GTACCTCTG	CCCTTACTGC	TCATCTGCTT	TTGCTATGCC	660
AAGGTCTTA	ATCATCTGCA	AAAAAAGCTG	AAAAACATGT	AAAAAAAGTC	TGAAGCATCC	720
AAGAAAAAGA	CTGCACAGAC	CGTCCTGGTG	GTGCGTTGAG	TATTTGGCAT	ATCCTGGCTG	780
CCCCATCATG	TCGTCCACCT	CTGGGCTGAG	TTTGGAGCCT	TCCCAC TGAC	GCCAGCTTCC	840
TTCTTCTTCA	GAATCACCGC	CCATTGCCTG	GCATACAGCA	ACTCCTCAGT	GAACCCCATC	900
ATATATGCCT	TTCTCTCAGA	AAACTTCCGG	AAGGGGTACA	AGCAAGTGT	TT CAAGTGTCA	960
GTGGCGATG	AATCTCCACG	CAGTGAAACT	AAGGAAAACA	AGAGCCGGAT	GGACACCCCG	1020
CCATCCACCA	ACTGCACCCA	CGTG				1044

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 125
(B) TYPE: Amino acid
(C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Leu Leu Thr Leu His Pro Val Trp Ser Gln Lys His Arg Thr Ser His
1 5 10 15

Trp Ala Ser Arg Val Val Leu Gly Val Trp Leu Ser Ala Thr Ala Phe
20 25 30

Ser Val Pro Tyr Leu Val Phe Arg Glu Thr Tyr Asp Asp Arg Lys Gly
35 40 45

Arg Val Thr Cys Arg Asn Asn Tyr Ala Val Ser Thr Asp Trp Glu Ser
50 55 60

Lys Glu Met Gln Thr Val Arg Gln Trp Ile His Ala Thr Cys Phe Ile
65 70 75 80

Ser Arg Phe Ile Leu Gly Phe Leu Leu Pro Phe Leu Val Ile Gly Phe

- 2 6 2 -

85

90

95

Cys Tyr Glu Arg Val Ala Arg Lys Met Lys Glu Arg Gly Leu Phe Lys
 100 105 110

Ser Ser Lys Pro Phe Lys Val Thr Met Thr Ala Val Ile
 115 120 125

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 377
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

CTTCTCACCC TTCACCCAGT GTGGTCCCAA AAGCACCGAA CCTCACACTG GGCTTCCAGA	60
GTCGTTCTGG GAGTCTGGCT CTCTGCCACT GCCTTCAGCG TGCCCTATTT GGTTTTCAGG	120
GAGACATATG ATGACCGTAA AGGAAGAGTG ACCTGCAGAA ATAAC TACGC TGTGTCCACT	180
GA C T G G A A A G C A A A G A G A T G C A A A C A G T A A G C A A T G G A T T C A T G C C A C C T G T T T C A T C	240
A G C C G C T T C A T A C T G G G C T T C C T T C T G C C T T T C T T A G T C A T T G G C T T T T G T T A T G A A A G A G	300
G T A G C C C G C A A G A T G A A A G A G A G G G G C C T C T T A A A T C C A G C A A A C C C T T C A A A G T C A C G	360
A T G A C T G C T G T T A T C T C	377

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 119
- (B) TYPE: Amino acid
- (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Phe Lys Ile Val Lys Pro Leu Ser Thr Ser Phe Ile Gln Ser Val Asn
 1 5 10 15

Tyr Ser Lys Leu Val Ser Leu Val Val Trp Leu Leu Met Leu Leu Leu
 20 25 30

Ala Val Pro Asn Val Ile Leu Thr Asn Gln Arg Val Lys Asp Val Thr
 35 40 45

- 2 6 3 -

Gln Ile Lys Cys Met Glu Leu Lys Asn Glu Leu Gly Arg Gln Trp His
50 55 60

Lys Ala Ser Asn Tyr Ile Phe Val Gly Ile Phe Trp Leu Val Phe Leu
65 70 75 80

Leu Leu Ile Ile Phe Tyr Thr Ala Ile Thr Arg Lys Ile Phe Lys Ser
85 90 95

His Leu Lys Ser Arg Lys Asn Ser Ile Ser Val Lys Lys Ser Ser
100 105 110

Arg Asn Ile Phe Ser Ile Val
115

(2) INFORMATION FOR SEQ ID NO: 51:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 357
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE
- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

TTCAAGATTG TGAAGCCCT TTCCACGTCC TTCATCCAGT CTGTGAACTA CAGCAAACTC 60
GTCTCGCTGG TGGTCTGGTT GCTCATGCTC CTCCTCGCCG TCCCCAACGT CATTCTCAC 120
AACCAGAGAG TTAAGGACGT GACGCAGATA AAATGCATGG AACTAAAAAA CGAACTGGGC 180
CGCCAGTGGC ACAAGGCGTC AAACTACATC TTTGTGGCA TTTTCTGGCT TGTGTTCCCT 240
TTGCTAATCA TTTTCTACAC TGCTATCACC AGGAAAATCT TTAAGTCCCA CCTGAAATCC 300
AGAAAAGATT CCATCTCGGT CAAAAAGAAA TCTAGCCGCA ACATCTTCAG CATCGTG 357

(2) INFORMATION FOR SEQ ID NO: 52:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 252
 - (B) TYPE: Amino acid
 - (C) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

Val Asp Leu Leu Ala Ala Leu Thr Leu Met Pro Leu Ala Met Leu Ser
1 5 10 15

- 2 6 4 -

Ser	Ser	Ala	Leu	Phe	Asp	His	Ala	Leu	Phe	Gly	Glu	Val	Ala	Cys	Arg	
							20				25			30		
Leu	Tyr	Leu	Phe	Leu	Ser	Val	Cys	Phe	Val	Ser	Leu	Ala	Ile	Leu	Ser	
							35				40			45		
Val	Ser	Ala	Ile	Asn	Val	Glu	Arg	Tyr	Tyr	Tyr	Val	Val	His	Pro	Met	
						50					55			60		
Arg	Tyr	Glu	Val	Arg	Met	Lys	Leu	Gly	Leu	Val	Ala	Ser	Val	Leu	Val	
						65					70			75		80
Gly	Val	Trp	Val	Lys	Ala	Leu	Ala	Met	Ala	Ser	Val	Pro	Val	Leu	Gly	
						85					90			95		
Arg	Val	Ser	Trp	Glu	Glu	Gly	Pro	Pro	Ser	Val	Pro	Pro	Gly	Cys	Ser	
							100				105			110		
Leu	Gln	Trp	Ser	His	Ser	Ala	Tyr	Cys	Gln	Leu	Phe	Val	Val	Val	Phe	
						115				120			125			
Ala	Val	Leu	Tyr	Phe	Leu	Leu	Pro	Leu	Leu	Ile	Leu	Val	Val	Tyr		
						130				135			140			
Cys	Ser	Met	Phe	Arg	Val	Ala	Arg	Val	Ala	Ala	Met	Gln	His	Gly	Pro	
						145				150			155		160	
Leu	Pro	Thr	Trp	Met	Glu	Thr	Pro	Arg	Gln	Arg	Ser	Glu	Ser	Leu	Ser	
						165				170			175			
Ser	Arg	Ser	Thr	Met	Val	Thr	Ser	Ser	Gly	Ala	Pro	Gln	Thr	Thr	Pro	
						180				185			190			
His	Arg	Thr	Phe	Gly	Gly	Lys	Ala	Ala	Val	Val	Leu	Leu	Ala	Val		
						195				200			205			
Gly	Gly	Gln	Phe	Leu	Leu	Cys	Trp	Leu	Pro	Tyr	Phe	Ser	Phe	His	Leu	
						210				215			220			
Tyr	Val	Ala	Leu	Ser	Ala	Gln	Pro	Ile	Ala	Ala	Gly	Gln	Val	Glu	Asn	
						225				230			235		240	
Val	Val	Thr	Trp	Ile	Gly	Tyr	Phe	Cys	Phe	Thr	Ser					
						245				250						

(2) INFORMATION FOR SEQ ID NO: 53:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 756
 - (B) TYPE: Nucleic acid
 - (C) STRANDEDNESS: Double
 - (D) TOPOLOGY: Linear

- (ii) MOLECULE TYPE: cDNA

- 2 6 5 -

(ix) FEATURE

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

GTGGACCTGC	TGGCTGCCCT	GACCCCATG	CCTCTGGCCA	TGCTCTCCAG	CTCCGCCCTC	60
TTTGACCACG	CCCTCTTGG	GGAGGTGGCC	TGCCGCCTCT	ACTTGTCCT	GAGCGTCTGC	120
TTTGTCAGCC	TGGCCATCCT	CTCGGTGTCC	GCCATCAATG	TGGAGCGCTA	CTATTATGTG	180
GTCCACCCCA	TGCGCTATGA	GGTGCATG	AAACTGGGGC	TGGTGGCCTC	TGTGCTGGTG	240
GGCGTGTGGG	TGAAGGCCCT	GGCCATGGCT	TCTGTGCCAG	TGTTGGGAAG	GGTGTCTGG	300
GAGGAAGGCC	CTCCCAGTGT	CCCCCAGGC	TGTTCACTCC	AATGGAGCCA	CAGTGCCTAC	360
TGCCAGCTTT	TCGTGGTGGT	CTTCGCCGTC	CTCTACTTCC	TGCTGCCCT	GCTCCTCATC	420
CTTGTGGTCT	ACTGCAGCAT	GTTCCGGGTG	GCTCGTGTGG	CTGCCATGCA	GCACGGGCCG	480
CTGCCAACGT	GGATGGAGAC	GCCCCGGCAA	CGCTCCGAGT	CTCTCAGCAG	CCGCTCCACT	540
ATGGTCACCA	GCTCGGGGGC	CCCGCAGACC	ACCCCTCACC	GGACGTTGG	CGGAGGGAAAG	600
GCAGCAGTGG	TCCTCCTGGC	TGTGGGAGGA	CAGTTCTGC	TCTGTTGGTT	GCCCTACTTC	660
TCCTTCCACC	TCTATGTGGC	CCTGAGCGCT	CAGCCCATTG	CAGCGGGGCA	GGTGGAGAAC	720
GTGGTGACCT	GGATTGGCTA	CTTCTGCTTC	ACCTCC			756

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 263
- (B) TYPE: Amino acid
- (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Ala	Asp	Val	Leu	Val	Thr	Ala	Ile	Cys	Leu	Pro	Ala	Ser	Leu	Leu	Val
1							5				10				15

Asp	Ile	Thr	Glu	Ser	Trp	Leu	Phe	Gly	His	Ala	Leu	Cys	Lys	Val	Ile
						20					25				30

Pro	Tyr	Leu	Gln	Ala	Val	Ser	Val	Val	Val	Leu	Thr	Leu	Ser		
							35			40			45		

Ser	Ile	Ala	Leu	Asp	Arg	Trp	Tyr	Ala	Ile	Cys	His	Pro	Leu	Leu	Phe
								50		55			60		

Lys	Ser	Thr	Ala	Arg	Arg	Ala	Arg	Gly	Ser	Ile	Leu	Gly	Ile	Trp	Ala
								65		70		75		80	

- 2 6 6 -

Val Ser Leu Ala Val Met Val Pro Gln Ala Ala Val Met Glu Cys Ser
 85 90 95

Ser Val Leu Pro Glu Leu Ala Asn Arg Thr Arg Leu Leu Ser Val Cys
 100 105 110

Asp Glu Arg Trp Ala Asp Asp Leu Tyr Pro Lys Ile Tyr His Ser Cys
 115 120 125

Phe Phe Ile Val Thr Tyr Leu Ala Pro Leu Gly Leu Met Ala Met Ala
 130 135 140

Tyr Phe Gln Ile Phe Arg Lys Leu Trp Gly Arg Gln Ile Pro Gly Thr
 145 150 155 160

Thr Ser Ala Leu Val Arg Asn Trp Lys Arg Pro Ser Asp Gln Leu Asp
 165 170 175

Asp Gln Gly Gln Gly Leu Ser Ser Glu Pro Gln Pro Arg Ala Arg Ala
 180 185 190

Phe Leu Ala Glu Val Lys Gln Met Arg Ala Arg Arg Lys Thr Ala Lys
 195 200 205

Met Leu Met Val Val Leu Leu Val Phe Ala Leu Cys Tyr Leu Pro Ile
 210 215 220

Ser Val Leu Asn Val Leu Lys Arg Val Phe Gly Met Phe Arg Gln Ala
 225 230 235 240

Ser Asp Arg Glu Ala Ile Tyr Ala Cys Phe Thr Phe Ser His Trp Leu
 245 250 255

Val Tyr Ala Asn Ser Ala Ala
 260

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 789
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

GCCGATGTGC TGGTGACAGC CATCTGCCCTG CCGGCCAGTC TGCTGGTAGA CATCACGGAA	60
TCCTGGCTCT TTGGCCATGC CCTCTGCAAG GTCATCCCCCT ATCTACAGGC CGTGTCCGTG	120

- 2 6 7 -

TCAGTGGTCG	TGCTGACTCT	CAGCTCCATC	GCCCTGGACC	GCTGGTACGC	CATCTGCCAC	180
CCGCTGTTGT	TCAAGAGCAC	TGCCCGGC	GCCCCGGC	GTGCTCGG	CATCTGGCG	240
GTGTCGCTGG	CTGTCATGGT	GCCTCAGGCT	GCTGTCATGG	AGTGTAGCAG	CGTGCTGCC	300
GAGCTGGCCA	ACCGCACCCG	CCTCCTGTCT	GTCTGTGATG	AGCGCTGGC	AGACGACCTG	360
TACCCCAAGA	TCTACCACAG	CTGCTTCTTC	ATTGTCACCT	ACCTGGCCCC	ACTGGGCCTC	420
ATGGCCATGG	CCTATTCCA	GATCTCCGC	AAGCTCTGGG	GCCGCCAGAT	CCCCGGCACC	480
ACCTCGGCC	TGGTGCGCAA	CTGGAAGCGG	CCCTCAGACC	AGCTGGACGA	CCAGGGCCAG	540
GGCCTGAGCT	CAGAGCCCCA	GCCCCGGG	CGCGCCTTCC	TGGCCGAGGT	GAAACAGATG	600
CGAGCCCCGA	GGAAAGACGGC	CAAGATGCTG	ATGGTGGTGC	TGCTGGTCTT	CGCCCTCTGC	660
TACCTGCCCA	TCAGTGTCC	CAACGTCTC	AAGAGGGTCT	TCGGGATGTT	CCGCCAAGCC	720
AGCGACCGAG	AGGCCATCTA	CGCCTGCTTC	ACCTTCTCCC	ACTGGCTGGT	GTACGCCAAC	780
AGCGCCGCC						789

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 328
- (B) TYPE: Amino acid
- (C) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Met	Glu	Trp	Asp	Asn	Gly	Thr	Gly	Gln	Ala	Leu	Gly	Leu	Pro	Pro	Thr
1				5						10				15	
Thr	Cys	Val	Tyr	Arg	Glu	Asn	Phe	Lys	Gln	Leu	Leu	Leu	Pro	Pro	Val
					20			25					30		
Tyr	Ser	Ala	Val	Leu	Ala	Ala	Gly	Leu	Pro	Leu	Asn	Ile	Cys	Val	Ile
					35			40				45			
Thr	Gln	Ile	Cys	Thr	Ser	Arg	Arg	Ala	Leu	Thr	Arg	Thr	Ala	Val	Tyr
					50		55			60					
Thr	Leu	Asn	Leu	Ala	Leu	Ala	Asp	Leu	Leu	Tyr	Ala	Cys	Ser	Leu	Pro
					65		70			75			80		
Leu	Leu	Ile	Tyr	Asn	Tyr	Ala	Gln	Gly	Asp	His	Trp	Pro	Phe	Gly	Asp
						85			90			95			
Phe	Ala	Cys	Arg	Leu	Val	Arg	Phe	Leu	Phe	Tyr	Ala	Asn	Leu	His	Gly
					100			105			110				

- 2 6 8 -

Ser	Ile	Leu	Phe	Leu	Thr	Cys	Ile	Ser	Phe	Gln	Arg	Tyr	Leu	Gly	Ile
							115		120				125		
Cys	His	Pro	Leu	Ala	Pro	Trp	His	Lys	Arg	Gly	Gly	Arg	Arg	Ala	Ala
							130		135			140			
Trp	Leu	Val	Cys	Val	Thr	Val	Trp	Leu	Ala	Val	Thr	Thr	Gln	Cys	Leu
							145		150			155			160
Pro	Thr	Ala	Ile	Phe	Ala	Ala	Thr	Gly	Ile	Gln	Arg	Asn	Arg	Thr	Val
							165		170			175			
Cys	Tyr	Asp	Leu	Ser	Pro	Pro	Ala	Leu	Ala	Thr	His	Tyr	Met	Pro	Tyr
							180		185			190			
Gly	Met	Ala	Leu	Thr	Val	Ile	Gly	Phe	Leu	Leu	Pro	Phe	Ala	Ala	Leu
							195		200			205			
Leu	Ala	Cys	Tyr	Cys	Leu	Leu	Ala	Cys	Arg	Leu	Cys	Arg	Gln	Asp	Gly
							210		215			220			
Pro	Ala	Glu	Pro	Val	Ala	Gln	Glu	Arg	Arg	Gly	Lys	Ala	Ala	Arg	Met
							225		230			235			240
Ala	Val	Val	Val	Ala	Ala	Ala	Phe	Ala	Ile	Ser	Phe	Leu	Pro	Phe	His
							245		250			255			
Ile	Thr	Lys	Thr	Ala	Tyr	Leu	Ala	Val	Gly	Ser	Thr	Pro	Gly	Val	Pro
							260		265			270			
Cys	Thr	Val	Leu	Glu	Ala	Phe	Ala	Ala	Ala	Tyr	Lys	Gly	Thr	Arg	Pro
							275		280			285			
Phe	Ala	Ser	Ala	Asn	Ser	Val	Leu	Asp	Rro	Ile	Leu	Phe	Tyr	Phe	Thr
							290		295			300			
Gln	Lys	Lys	Phe	Arg	Arg	Arg	Pro	His	Glu	Leu	Leu	Gln	Lys	Leu	Thr
							305		310			315			320
Ala	Lys	Trp	Gln	Arg	Gln	Gly	Arg								
															325

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 984
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE

- (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

- 2 6 9 -

ATGGAATGGG ACAATGGCAC AGGCCAGGCT CTGGGCTTGC CACCCACCAC	60
CGCGAGAACT TCAAGCAACT GCTGCTGCCA CCTGTGTATT CGGCGGTGCT GGCGGCTGGC	120
CTGCCGCTGA ACATCTGTGT CATTACCCAG ATCTGCACGT CCCGCCGGGC CCTGACCCGC	180
ACGGCCGTGT ACACCCCTAAA CCTTGCTCTG GCTGACCTGC TATATGCCTG CTCCCTGCC	240
CTGCTCATCT ACAACTATGC CCAAGGTGAT CACTGGCCCT TTGGCGACTT CGCCTGCC	300
CTGGTCCGCT TCCTCTTCTA TGCCAACCTG CACGGCAGCA TCCTCTTCCT CACCTGCATC	360
AGCTTCCAGC GCTACCTGGG CATCTGCCAC CCGCTGGCCC CCTGGCACAA ACGTGGGGC	420
CGCCGGGCTG CCTGGCTAGT GTGTGTAACC GTGTGGCTGG CCGTGACAAC CCAGTGCCTG	480
CCCACAGCCA TCTTCGCTGC CACAGGCATC CAGCGTAACC GCACTGTCTG CTATGACCTC	540
AGCCCCGCTG CCCTGGCAC CCACTATATG CCCTATGGCA TGGCTCTCAC TGTCACTGGC	600
TTCCTGCTGC CCTTTGCTGC CCTGCTGGCC TGCTACTGTC TCCTGGCCTG CCGCCTGTGC	660
CGCCAGGATG GCCCCGGCAGA GCCTGTGGCC CAGGAGCGGC GTGGCAAGGC GGCCCGCATG	720
GCCGTGGTGG TGGCTGCTGC CTTGCCATC AGCTTCCTGC CTTTTCACAT CACCAAGACA	780
GCCTACCTGG CAGTGGGCTC GACGCCGGGC GTCCCCTGCA CTGTATTGGA GGCCTTGCA	840
GCGGCCTACA AAGGCACGCG GCCGTTGCC AGTGCCAAC ACGTGCTGGA CCCCATCCTC	900
TTCTACTTCA CCCAGAAGAA GTTCCGCCGG CGACCACATG AGCTCCTACA GAAACTCACA	960
GCCAAATGGC AGAGGCAGGG TCGC	984

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

ACAGCCATCT TCGCTGCCAC AGGCAT 26

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29
- (B) TYPE: Nucleic acid
- (C) STRANDEDNESS: Single

- 2 7 0 -

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

AGACAGTAGC AGGCCAGCAG GGCAGAAA 29

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

CTGTGYYGSA TYGCNNTKGA YMGSTAC 27

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29
(B) TYPE: Nucleic acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid
Synthetic DNA

(iii) FEATURES: N is inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

AKGWAGWAGG GCAGCCAGCA GANSRYGAA 29

CLAIMS

1. A DNA which comprises a nucleotide sequence represented by a SEQ ID NO selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 19.

5 2. A method for amplifying a DNA coding for a G protein coupled receptor protein by polymerase chain reaction techniques, which comprises:

(i) carrying out a polymerase chain reaction in the presence of a mixture of

10 ① a DNA coding for a G protein coupled receptor protein, said DNA being capable of acting as a template,

② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and

15 ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 9, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide

- 2 7 2 -

sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19; or

5 (ii) carrying out a polymerase chain reaction in the presence of a mixture of

- ① a DNA coding for G protein coupled receptor protein, said DNA being capable of acting as a template,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13.

3. A method for screening a DNA library for a DNA coding for a G protein coupled receptor protein, which comprises:

20 (i) carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library,
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 3, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 5, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 6, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 7, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 10, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 14, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 16 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 18, and
- ③ at least one DNA primer selected from the group

- 2 7 3 -

consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 2, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 4, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 8, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 11, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 15, DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 17 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 19,

under conditions to amplify selectively a template DNA coding for the G protein coupled receptor protein, contained in the DNA library and selecting said DNA; or

(ii) carrying out a polymerase chain reaction in the presence of a mixture of

- ① said DNA library
- ② at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 1 and DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 12, and
- ③ at least one DNA primer selected from the group consisting of DNA primers comprising a nucleotide sequence represented by SEQ ID NO: 13,

under conditions to amplify selectively a DNA coding for the G protein coupled receptor protein, contained in the DNA library and selecting said DNA.

4. A DNA coding for a G protein coupled receptor protein or a fragment thereof, which is obtained by the method according to claim 2 to 3.

5. A G protein coupled receptor protein encoded by the DNA according to claim 4, a peptide segment or fragment thereof or a salt thereof.

6. A G protein coupled receptor protein comprising an amino acid sequence selected from the group consisting of

- 2 7 4 -

an amino acid sequence represented by SEQ ID NO: 24, an amino acid sequence represented by SEQ ID NO: 25, an amino acid sequence represented by SEQ ID NO: 26, an amino acid sequence represented by SEQ ID NO: 27, an amino acid sequence
5 represented by SEQ ID NO: 28, an amino acid sequence represented by SEQ ID NO: 34, an amino acid sequence represented by SEQ ID NO: 35, an amino acid sequence represented by SEQ ID NO: 38, an amino acid sequence represented by SEQ ID NO: 39, an amino acid sequence
10 represented by SEQ ID NO: 56, and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 38, SEQ ID NO: 39, or SEQ ID NO: 56; a peptide segment (or fragment) thereof, a modified peptide
15 derivative thereof or a salt thereof.

7. The G protein coupled receptor protein according to claim 6, comprising an amino acid sequence selected from the group consisting of an amino acid sequence represented by SEQ ID NO: 38, an amino acid sequence represented by SEQ ID NO: 39, an amino acid sequence represented by SEQ ID NO: 56 and substantial equivalents to the amino acid sequence represented by SEQ ID NO: 38, SEQ ID NO: 39, or SEQ ID NO: 56.

8. The G protein coupled receptor protein according to claims 6 or 7, wherein said receptor is a purinoceptor.

9. The G protein coupled receptor protein according to any of claims 6 to 8, wherein an agonist to said receptor is useful as an immunomodulator or an antitumor agent, in addition it is useful in therapeutically or prophylactically
30 treating hypertension, diabetes or cystic fibrosis, and an antagonist to said receptor is useful as a hypotensive agent, an analgesic, or an agent for therapeutically or prophylactically treating incontinence of urine.

10. A DNA which comprises a nucleotide sequence coding for a G protein coupled receptor protein of claim 6.

11. The DNA according to claim 10 comprising a nucleotide sequence coding for the G protein coupled receptor

- 2 7 5 -

protein according to claim 7.

12. The DNA according to claim 11 comprising a nucleotide sequence represented by SEQ ID NO: 40, SEQ ID NO: 41, or SEQ ID NO: 57.

5 13. A transformant containing a vector comprising the DNA according to claim 4 or 10; or an expression system comprising an open reading frame (ORF) of DNA derived from a G protein coupled receptor protein DNA according to claim 4 or 10, wherein the ORF is operably linked
10 to a control sequence compatible with a desired host cell.

14. A method for determining a ligand to the G protein coupled receptor protein according to any of claims 5 to 8, which comprises contacting

15 (i) at least one component selected from the group consisting of G protein coupled receptor proteins or salts thereof according to any of claims 5 to 8,
peptide segments or salts thereof, and mixtures thereof,
with

20 (ii) at least one compound to be tested
and determining whether said compound to be tested bound to the component of (i).

15. A screening method for a compound capable of inhibiting the binding of a G protein coupled receptor protein according to any of claims 5 to 8 with a ligand, which
25 comprises carrying out a comparison between:

(i) at least one case where said ligand is contacted
with at least one component selected from the group
consisting of G protein coupled receptor proteins or
salts thereof according to any of claims 5 to 8,
30 peptide segments or salts thereof, and mixtures thereof,
and

35 (ii) at least one case where said ligand together
with a compound to be tested is contacted with at
least one component selected from the group consisting
of G protein coupled receptor proteins or salts thereof
according to any of claims 5 to 8, peptide segments or
salts thereof, and mixtures thereof.

- 2 7 6 -

16. A compound which is determined through the method according to claim 15 or a salt thereof.

17. The compound according to claim 16, which is an agonist or antagonist to a G protein coupled receptor protein
5 according to any of claims 5 to 8.

18. A ligand to a G protein coupled receptor protein according to any of claims 5 to 8, which is determined through the method according to claim 14.

10

15

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FIGURE 1

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer HS-1	CGTGGCCATCCTGGGCAACACCCCTG
	G C GG
	CT
	G
	T

HTRHR	CCTGGGCATTGTAGGCAACATCATGGT
HUMRANTES	CATTGGCCTGGTTGGAAACATCCTGGT
HSBLR1A	CCTGGGCCTGATCGGCAACGTCCCTGGT
HUMSOMAT	GGTGGGGCTGGTGGGCAACGCCCTGGT
RNU02083	AGTGGGCCTTCGGAAACTTCCTGGT
U00442	GGTGGGCTTAGTGGCAATTCCCTGGT
HUMNMBR	CGTGGGCTTGCTGGCAACATCATGCT
HSHM4	GGTGACCATCATCGGCAACATCCTGGT
RATAADRE01	CTTGCCATCGTGGCAACATCTGGT
HUMSSTR3X	GGTGGGCCTGCTGGTAACTCGCTGGT
HUMC5AAR	GGTGGGAGTGCTGGCAATGCCCTGGT
HUMRDC1A	CATCGGCATGATTGCCAACCTCCGTGGT
HUMOPIODRE	CGTGGCGGTGCTCGGCAACCTCGTGGT
RATA2BAR	GCTGGCAGTGGCGGGCAACGTGCTGGT

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FIGURE 2

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 3' SIDE PRIMER

Complementary Sequence to Primer HS-2	TTTGCATCTGGATGCCAAC
	C C TTT C
	G G
	T T

HUMSGIR	TTTGCCCTCTGGTCCCTCAAC
HUMBOMB3S	TTTGCCCTCTGGTGCCTACAC
S46950	TTTGCCCTCTGGCTGCCCTACAC
MUSGPCR	TTTGCCCTCGTCTGGTGCCTCTCAAC
S43387	TTTGCCCTTTATGGATGCCCTACAGG
RATNEURA	TTTGCCATCTGGCTGCCCTATCAC
RATA1ARA	TTTGCCCTCAGCTGGCTGCCGCTGCAT
HUMOPIODRE	TTTGCCATCTGGCTGCCCTATCAC
HUMNEKAR	TTTGCCATCTGGCTGCCCTACAC
RATADENREC	TTTGCCCTGTGCTGGCTGCCCTTGTCC
HUMSRI1A	TTTGTCATCTGGATGCCCTTCTAC
S8637154	TTTGCTATCTGGCTGCCCTATCAT
RNCGPCR	TTTGCCGCTGGATGCCCTTACC
HUMSSTR4Z	TTTGTGCTCTGGATGCCCTTCTAC
RATGNRHA	TTTGACACTGGTCGAAGCCAGACAA

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FIGURE 3

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer 3A

CTGACCGCTCTIACIACTGACCGATA
T T GG GT A C
 G

Primer 3B

CTGACCGCTCTIACIACTGACCGATA
T T GG GT A C
 G

L11064

CTCACCATGATGAGCGTGGACCGCTAC

L11065

TTGACCATGATGGAGTGTGACCGCTAC

D16349

CTCTGCACCATGAGCGTGGACCGCTAC

X69676

CTGATGCTCGTGAGTATCGACCGCTAC

M35328

CTTACGGCACTGTCAGCTGACAGGTAC

M73482

CTCACTGCCCTCAGCGCCGACAGGTAC

M73481

CTCACGGCGCTCTCGGCAGACAGATAAC

L08893

TTAACAAATTCTCAGCGCTGACAGATAAC

X62933

ATGACCGCCATCGCCGCTGACAGGTAC

X62934

ATGACAACGTGGCCTTGACAGATAAC

J05189

ATGACAGCCATTGCAGTGGACAGGTAT

M60786

CTCTGCCTCTCAGTGTGGACAGGTAC

L04672

CTCACCTGCCTCAGCATTGACCGCTAC

X61496

TTGCTGGCTATCACTGTGGACCGCTAC

X59249

TTGCTGGCCATTGCTGTAGACCGATAAC

L09249

CTCACCTGCCTCAGCATTGACCGCTAC

P30731

CTGACAGCTATCGCAGTGGACCGCCAC

M31210

CTCCTGCCATGCCATTGAGCGCTAT

U03642

CTCACCGGCCTCAGCTTCGACCGCTAC

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FIGURE 4

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer 3C

CTCGCCGCTATIAGCATGGACCGITAC
G CC G T T

Primer 3D

CTCGCCGCTATIAGCATGGACCGITAT
G CC G T T

L32840

ATTACCTGCATGAGTGTGATAGGTAC

X64052

CTCACGTGTCTCAGCATCGATCGCTAC

M90065

CTCACGTGTCTCAGCATCGATCGCTAC

M91464

CTCACGTGTCTCAGCATTGATCGATAAC

M88096

CTGGTAGGCCATCTCTGGAGAGATAT

M99418

CTCGTGGCCATAGCCCTGGAGCGATAAC

L04473

CTCGTGGCCATCGCACTGGAGCGGTAC

M73969

CTGGCCTGCATCAGTGTGGACCGTTAC

X65858

TTGGCCTGCATCAGTGTGGACCGTTAC

S46665

CTGGCTACCATTAAGTGCCGACCGTTTC

M60626

ATCGCCCTCATTGCTCTGGACCGCTGT

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FIGURE 5

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 3' SIDE PRIMER

Complementary Sequence TTTACCITCTGTTGGICGCCCTACCAATC
to Primer 6A GT TC T T

Complementary Sequence TTCACCITCTGTTGGICGCCCTACCAATC
to Primer 6B GT TC T T

L11064	TTCGTGGTGTGCTGGCGCCATCCACATC
L11065	TTCATCATCTGTTGGACCCCCATTACATC
D16349	TTTATCGTCTGCTGGACCCCCATCCACATC
X69676	TTTGTGCTGTGTTGGGTGCCTTCCAGATC
M35328	TTTGCCTTCTGCTGGCTCCCCAACCATGTC
M73482	TTCATCTTCTGTTGGTTCCAACACATC
M73481	TTCGCCTTCTGCTGGCTCCCCAATCATGTC
L08893	TTTGCCCTCTGCTGGTTGCCAAATCACCTC
X62933	TTTGCCATCTGCTGGCTGCCCTACCACCTC
X62934	TTCGCCATCTGCTGGCTGCCCTACACATC
J05189	TTTGCCATCTGCTGGCTGCCCTATCACGTG
M60786	TTCGCCCTGTGCTGGTTCCCTTCACTTA
L04672	TTTGTCATCTGCTGGCTGCCCTACACGTG
X61496	TTTGCCGCTCTGCTGGATGCCCTTACCCCTC
X59249	TTTGCCCTGTGCTGGCTGCCCTTGTCCATC
L09249	TTTGCCATCTGCTGGCTGCCCTACACGTG
P30731	TTTGCCCTCTGCTGGTTCCCTCTCAACTGC
M31210	TTCATGCCTGCTGGGCACCGCTTTCATC
U03642	TTTGCCCTGTGCTGGATGCCCTACCACCTG

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FIGURE 6

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 3' SIDE PRIMER

Complementary Sequence TTTTCITTGCTGGITTCCCTACACATG
to Primer 6C CC T G C T T

L32840	TTCATCATTGCTGGCTCCCTTCCATGTT
X64052	TTCTTCTTTCTGGGTTCCCCACCAAATA
M90065	TTCTTCTTTCTGGGTTCCCCACCAAATA
M91464	TTTTCTTTCTGGATTCCCCACCAAATA
M88096	TTCTTCCTGTGCTGGATGCCATCTTCAGC
M99418	TTCTTCCTGTGTTGGCTGCCAGTGTACAGC
L04473	TTTTTCTGTGTTGGTTGCCAGTTATAGT
M73969	TTCCCTGCTTGCTGGCTGCCCTACAACCTG
X65858	TTCCCTGCTTGCTGGCTGCCCTACAACCTG
S46665	TTCTTTATCTTCTGGCTGCCCTATCAGGTG
M60626	TTTTTCTGTGCTGGTCCCCATATCAGGTG

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FIGURE 7

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer T2A

GTCACCAACITGTTCATCCTCAICCTG
C AC GT T
 A

HUMGALAREC	ACCACCAACCTGTTCATCCTCAACCTG
RATADRA1B	CCCACCAACTACTTATCGTCAACCTG
HUMADRB1	ACCACCAACCTGTTCATCCTCAACCTG
RABIL8RSB	GTCACCGACGTCTACCTGCTGAACCTG
HUMOPIODRE	GTCACCAACTCCTCCTCGTGAACCTG
BTSKR	GTGACCAACTACTTATCGTCAACCTG
HUMSRI2A	ATCACCAACATTACATCCTCAACCTG
HUMSSTR3Y	GTCACCAACGTCTACATCCTCAACCTG
HUMGARE	GTCACCAACGCCTCCTCCTCTCACTG
HUMCCKAR	GTCACCAACATCTCCTCCTCTCCCTG
HUMSHTR	CCCTCCAACTAACCTGATCGTGTCCCTG
HUMD1B	ATGACCAACGTCTTATCGTGTCTCTG
HUM5HT1E	CCTGCCAACACTACCTAATCTGTTCTCTG
HUMD4C	CCCACCAACTCCTCATCGTGAGCCTG
MMSERO	GCCACCAACTATTCCTGATGTCACTT
RATADRA1A	GTCACCAACTATTCATCGTGAACCTG
S57565	CTGACCAATTGCTTCATTGTGTCCCTG

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FIGURE 8

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence AACCCCITCITCTATTGCTTTTCICT
to Primer T7A T T C C C G G

HUMGALAREC	AATCCTATCATTTATGCATTTCTCTCT
RATA1ADREC	AACCCCATCGTCTATGCCCTCCGGATC
PIGA2R	AATCCTCTCTTTATGGCTTCTGGGG
RAT5HRTC	AACCCATCATCTACCCGCTCTTATG
S58541	AACCCCATCATTTATGCCCTTAATGCT
HUMGRPR	AACCCCTTGCCTCTACCTGCTGAGC
MUSGRPBOM	AACCCCTTGCTCTTATCTGCTGAGC
RRVT1AIIR	AACCCCTCTGTTCTACGGCTTCTGGGG
HUMADRB1	AACCCCATCATCTACTGCCGCAGCCCC
HSHM4	AACCCCGTGTGCTATGCTCTGTGCAAC
HUMGARE	AACCCCTGGTCTACTGCTTCATGCAC
RATCCKAR	AACCCCATCATCTATTGCTTCATGAAC
S59749	AATCCCATGCTCTACACCTCGCTGGC
HUMSST28A	AACCCCGTCTACGGCTTCTCTCG
RNGPROCR	AACCCCATCCTCTACGGCTTCCCTCTCC
MUSSSRI1A	AACCCCATACTCTACGGCTTCTGTGCG
HUMA1AADR	AACCCGCTCATCTACCCCTGTTCCAGC
S66181	AACCCGGTTCTACGCCTTCCCTGGAC
HUMSSTR3Y	AACCCCATCCTTATGGCTTCCCTCTCC

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FIGURE 9

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer TM1-A2 TGITGGTTATIGGIGTTGTIGGIAA
 CC GC C G

MUSB2R	TGGTGGTGGTGGTGGTGGTGGCAA
BTSKR	TGGTGCTGGTGGCTGTGATGGCAA
BOVEETBR	TGTCGTGCTGGGCATCATCGGAAA
HUMNEUYREC	TGATCATTCTTGGTGTCTCTGGAAA
MMSUBKREC	TGGTGCTGGTGGCTGTAACAGGCAA
HUMPGE2R	TGTTCATCTCAGGGGTGGTGGCAA
HUMPIR	TGTCGTGGCCGGTGTGGTGGCAA
HSU11053	TGTCGTGCTGGCTTGGTGGCAA
RRMC3RA	TGGTGATCCTGGCTGTGGTGAGGAA
HUMMR	TGGTTATCCTGGCCGTGGTCAGGAA
MUSGRPBM	TCATCGTGTAGGTCTTATTGGCAA
RATCHOLREC	TCTTCTGATGAGTGTGGCGGAAA
RATCCKAR	TATTCCTCTCAGTGTGCAGGGGAA

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FIGURE 10

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence GCCATIACCITGGACAGATAACCGAT
to Primer TM3-B2 A T A C G A G

HUMCCKR	GCCATCGCACTGGAGCGGTACAG
HUMCCKBGR	GCCATCGCACTGGAGCGGTACAG
MMGMC5R	GCCATTGCGGTGGACAGGTACA
HUMV2R	GCCATGACGCTGGACCGCCACCG
RATNEURA	GCCATTGCAGTGGACAGGTA
DOGGSTRN	GCCATCGCCCTGGAGCGATAACAG
RAT5HT5A	GCAATAGCTTGGACCGCTACTGGT
MUSALP2ADA	GCCATTAGTCTGGACCGCTACTGGT
HUMADORA1X	GCAATTGCTGTGGACCGCTACC
HUMOPIODRE	GCCATCGCGGTGGACAGATAACA
MUSGRPBM	GCACTGTCAGCTGACAGGTACAAA
RATCCKAR	GCCATCTCTCTGGAGAGATATGG
HSTRHREC	GCCTTACCATTGAGAGGTACATA

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FIGURE 11

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer TM3-C2 CATGGCCGTGGAGAGITACITGGC
 TT C C T A

HUMNK3R	CATTGCGGTGGACAGGTATATGGC
HSMRNAOXY	CATGTCCCTGGACCGCTGCCTGGC
S68242	CATATCGCTGGAGAGATAACGGAGC
CFGPCR4	CATCGCTCTGGACAGGTACTGGGC
MMSUBPREC	TGGCCTTGACAGATAACATGGC
HUMOPIODRE	CATCGCGGTGGACAGATAACATGGC
HUMGALAREC	ATGTCCGTGGACCGCTACGTGGC
HSS31G	CATTGCCCTGGACAGGTACTGGGC
HUMARB3A	CCTGGCCGTGGACCGCTACCTGGC
HUMHPR	CATGGCCGTGGAGCGCTGCCTGGC
RATCCKAR	CATCTCTGGAGAGATAATGGCGC

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FIGURE 12

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence TTTGCCITCTGCTGGATCCCCAAC
to Primer TM6-E2 C G C G TT

HUMNEKAR	TTTGCCATCTGCTGGCTGCCCTAC
HUMSUBPRA	TTCGCCATCTGCTGGCTGCCCTTC
RATSKR	TTTGCCATCTGCTGGCTGCCCTAC
MUSGRPBM	TTTGCCCTCTGCTGGCTCCCCAAC
HUMOPIODRE	TTTGCCATCTGCTGGCTGCCCTA
HUMA2XXX	TTTGCCCTCTGCTGGCTGCCCT
HUMADRBR	TTCACCCCTCTGCTGGCTGCCCTTC
CFGPCR8	TTCGCCCCCTCTGCTGGCTGCCCT
HUMETSR	TTTGCCCTCTGCTGGCTTCCCCCT
MMNPY1CDS	TTCGCCGTCTGCTGGCTGCCCT
HSMRNAOXY	TTCATCGTGTGCTGGACGCCCTTC
RATCCKAR	TTCTTCTGTGCTGGATGCCATC

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FIGURE 13

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer TM2F18

ARYYTIGCIITIGCNGAY

HUMTSHX	AACCTGGCCTTGCGGAT
HUMNEKAR	AATCTGGCGCTGGCTGAC
HUMFMLP	AACCTGGCCGTGGCTGAC
HUMINTLEU8	AACCTAGCCTTGGCCGAC
HUMA1AADR	AACCTGGCCGTGGCCGAC
HUMIL8RA	AACCTGGCCTTGGCCGAC
HSDD2	AGCCTCGCAGTGGCCGAC
HUMANTIR	AATTAGCACTGGCTGAC
HUMSOMAT	AACCTGGCCGTAGCCGAC
HUMEL4REC	AGCTTGGCTGTGGCTGAT
HSTRHREC	AGCCTGGCAGTAGCTGAT
HSU07882	AACCTGGCCTTAGCCGAT

(R = A or G, Y = C or T, N = A, C, G or T, and
I = Inosine)

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FIGURE 14

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence TTYNYNNTNTGYTGGITICCI
to Primer TM6R21

HSBAR	TTCACCCCTCTGCTGGCTGCC
HUMNEKAR	TTTGCCATCTGCTGGCTGCC
HUMETN1R	TTTGCTCTTGCTGGTTCCCT
HUMHISH2R	TTCATCATCTGCTGGTTCC
HUMA1AADR	TTCGTGCTCTGCTGGTTCC
HUMIL8RA	TTCCCTGCTTGCTGGCTGCC
HUMNMBR	TTCATCTTCTGTTGGTTCC
HUMNKIRX	TTCGCCATCTGCTGGCTGCC
HUMSUBPRA	TTCGCCATCTGCTGGCTGCC
HUM5HT1DA	TTTATCATCTGCTGGCTGCC
HUMPFPR2A	TTCTTCATCTGTTGGTTCC
HSDD2	TTCATCATCTGCTGGCTGCC
HUMNEUYREC	TTTGCAGTCTGCTGGCTCC
HUM2XXX	TTTGCCTCTGCTGGCTGCC
HUMBK2A	TTCATCATCTGCTGGCTGCC
HUMFMLPX	TTCTTCATCTGTTGGTTCC
HUMSSTR3X	TTCGTGCTCTGCTGGATGCC
HUMCCKR	TTTTTCTGTGTTGGTTGCC
HSNEURA	TTTGTGGTCTGCTGGCTGCC

(Y = C or T, N = A, C, G or T, and I = Inosine)

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FIGURE 15

OLIGODEOXYNUCLEOTIDE SEQUENCE FOR 5' SIDE PRIMER

Primer S3A

GCCTGITAIGATGAGTGTGGAIAGIT
C G C T C C

HUMGALAREC
S70057
S67127
S44866
HUMC5AAR
HUMANTIR
HUMBK2A
HSNEURA
HUMGRPR
HUMFSRS
HUMIL8RA
HUMNEKAR

CCCTGGCCCGCATGCCGTGGACCGCT
GCCTCGTGGCCATCGCACTGGAGCGGT
ACCTCTGCGCTCTTAGTGTGACAGGT
GTCTATGTGCTCTGAGTATTGACAGAT
TCCTGGCCACCATCAGCGCCGACCGCT
TAATCACGTGTCTCAGCATTGATCGAT
TCCTGATGCTGGTGAGCATCGACCGCT
ACGTGGCCAGCCTGAGTGTGGAGCGCT
CACTCACGGCGCTCTGGCAGACAGAT
GCCTGACAGTCATGAGCGTGGACCGCT
TGTTGGCCTGCATCAGTGTGGACCGTT
CCATGACCGCCATTGCTGCCGACAGGT

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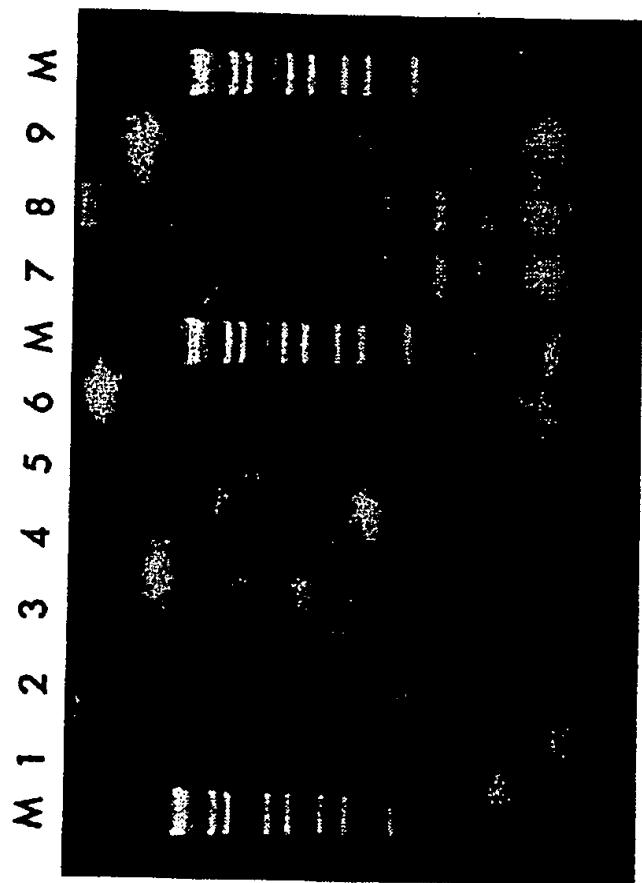
FIGURE 16

COMPLEMENTARY OLIGODEOXYNUCLEOTIDE SEQUENCE TO 3' SIDE PRIMER

Complementary Sequence TGGITICCCTACCACITIATCAICATC
to Primer S6A T T GG GT

HUMGALAREC	TGGCTGCCGCACCATCATCCATCTC
S70057	TGGTTGCCAGTTATAGTGCCAACACG
S67127	TGGTTCCCTCTTCATTAAAGCCGTATA
S44866	TGGCTTCCCCCTTCACCTCAGCAGGATT
HUMC5AAR	TGGTTGCCCTACCAGGTGACGGGGATA
HUMANTIR	TGGATTCCCCACCAAATATTCACTTT
HUMBK2A	TGGCTGCCCTCCAGATCAGCACCTTC
HSNEURA	TGGACTCCGTTCTCTATGACTTCTAC
HUMGRPR	TGGCTCCCAATCATGTCTACCTG
HUMFSRS	TGGCTGCCCTCTTCACCGTCAACATC
HUMIL8RA	TGGCTGCCCTACAACCTGGTCCTGCTG
HUMNEKAR	TGGCTGCCCTACCACCTCTACTTCATC

FIGURE 11



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FIGURE 18

		10	20	30	40	
A58-T7-2		<u>CTCGGCATGTCGGCAACCCCTGGTCACTTCCTGATCCTTCGCTACGC</u>				
HUMSOMAT	X:::	X:::GTGCGGCTGGTGGCAACGCCCTGGTCACTTCCTGATCCTTCGCTACGC				
		285	295	305	315	325
		60	70	80	90	100
A58-T7-2		CAAGATGAAGACGGCTACCAACATCTACCTGCTCAACCTGGCGTAGCCG				
HUMSOMAT	:::::	:::::CAAGATGAAGACGGCTACCAACATCTACCTGCTCAACCTGGCGTAGCCG				
		335	345	355	365	375
		110	120	130	140	150
A58-T7-2		ACGAGCTCTCATGCTGAGCGTGCCTTCCTGGCCCTGTCGCCCCCTG				
HUMSOMAT	:::::	:::::ACGAGCTCTCATGCTGAGCGTGCCTTCCTGGCCCTGTCGCCCCCTG				
		385	395	405	415	425
		160	170	180	190	200
A58-T7-2		CGCCACTGGCCTTCGGTCCGGTCCGGGCGGGCTGCTCAGCGTCA				
HUMSOMAT	:::::	:::::CGCCACTGGCCTTCGGTCCGGTCCGGGCGGGCTGCTCAGCGTCA				
		435	445	455	465	475
		210	220	230	240	
A58-T7-2		CGGCCCTAACATGGTCAACCAGCGTCTCTGCTCACCGTGCTCAGCGT				
HUMSOMAT	:::::	:::::CGGCCCTAACATGGTCAACCAGCGTCTCTGCTCACCGTGCTCAGCGT				X
		485	495	505	515	

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FIGURE 19

A58-SP6		10	20	30	40	50
HUMSOMATA	CAGTGTCACACCCGGCCTGGTCCAGTCCTCTGCTTACACTTTCCT					
	X:: :::: :::: :::: :::: :::: :::: :::: :::: :::					
	CAGTGCCACACCCGGCCTGGTCCAGTCCTCTGCTTACACTTTCCT					
	706	716	726	736	746	
A58-SP6	60	70	80	90	100	
HUMSOMATA	GCTGGCTTCCTGCTGTCCGTCCTGCCTGGCATTTGGCCTGCTACCTGCTCA					
	:::: :::: :::: :::: :::: :::: :::: :::: :::					
	GCTGGCTTCCTGCTGTCCGTCCTGGCATTTGGCCTGCTACCTGCTCA					
	756	766	776	786	796	
A58-SP6	110	120	130	140	150	
HUMSOMATA	TGTTGGCAAGATGCCGCCGTGTCCTGGCGCTGGCTGGCAGGAGCGC					
	:::: :::: :::: :::: :::: :::: :::: :::					
	TGTTGGCAAGATGCCGCCGTGTCCTGGCGCTGGCTGGCAGGAGCGC					
	806	816	826	836	846	
A58-SP6	160	170	180	190	200	
HUMSOMATA	AGGGCTGGAGAAAATCACCAAGGCTGGCTGCTGATGGCTGGCTGGT					
	:::: :::: :::: :::: :::: :::: :::					
	AGGGCTGGAGAAAATCACCAAGGCTGGCTGCTGATGGCTGGCTGGT					
	856	866	876	886	896	
A58-SP6	210	220				
HUMSOMATA	<u>CTTGGCCCTCTGCTGGTGCCTCCAC</u>					
	:::: :::: :::: :::: :::: :::: :::					
	CTTGGCTCTGCTGGATGCCCTTCTAC					
	906	916				

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FIGURE 20

		10	20	30	40	50
57-A-2		GTGGGCATGCTGGGCAACCTCCCTGGAAGGCAGT CGCGAGGTGGCCGGTT				
HUMDRD5A		X:::	:::::	:::::	:::::	:::::
		GTGGCGCTGCTGGTCATGC-CCTGGAAGGCAGT CGCGAGGTGGCCGGTT				
		424	434	444	454	
		60	70	80	90	100
57-A-2		ACTGGCCCTTGGAGCGTCTGCGACGTCTGGGTGGCCTTCGACATCATG				
HUMDRD5A		:::::	:::::	:::::	:::::	:::::
		ACTGGCCCTTGGAGCGTCTGCGACGTCTGGGTGGCCTTCGACATCATG				
		464	474	484	494	504
		110	120	130	140	150
57-A-2		TGCTCCACTGCTOCATCCTGAAACCTGTGCGTCACTAGCGTGGACCGCTA				
HUMDRD5A		:::::	:::::	:::::	:::::	:::::
		TGCTCCACTGCTOCATCCTGAAACCTGTGCGTCACTAGCGTGGACCGCTA				
		514	524	534	544	554
		160	170	180	190	200
57-A-2		CTGGGCCATCTCCAGGCCCTTCGGCTACAAGCGCAAGATGACTCAGCGCA				
HUMDRD5A		:::::	:::::	:::::	:::::	:::::
		CTGGGCCATCTCCAGGCCCTTCGGCTACAAGCGCAAGATGACTCAGCGCA				
		564	574	584	594	604
		210	220	230	240	250
57-A-2		TGGCCTTGGTCATGGTCGGCTGGCATGGACCTTGTCCTCATCTCATCTCC				
HUMDRD5A		:::::	:::::	:::::	:::::	:::::
		TGGCCTTGGTCATGGTCGGCTGGCATGGACCTTGTCCTCATCTCATCTCC				
		614	624	634	644	654
		260	270	280	290	300
57-A-2		TTCATTCCGGTCCAGGTCAACTGGACAGGGACCAGGGGGCTTGGGG				
HUMDRD5A		:::::	:::::	:::::	:::::	:::::
		TTCATTCCGGTCCAGGTCAACTGGACAGGGACCAGGGGGCTTGGGG				
		664	674	684	694	704
		310				
57-A-2		GGGGCTGGACCTGCCAAA				
HUMDRD5A		:::::	X			
		CGGGCTGGACCTGCCAAA				
		714	724			

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FIGURE 21

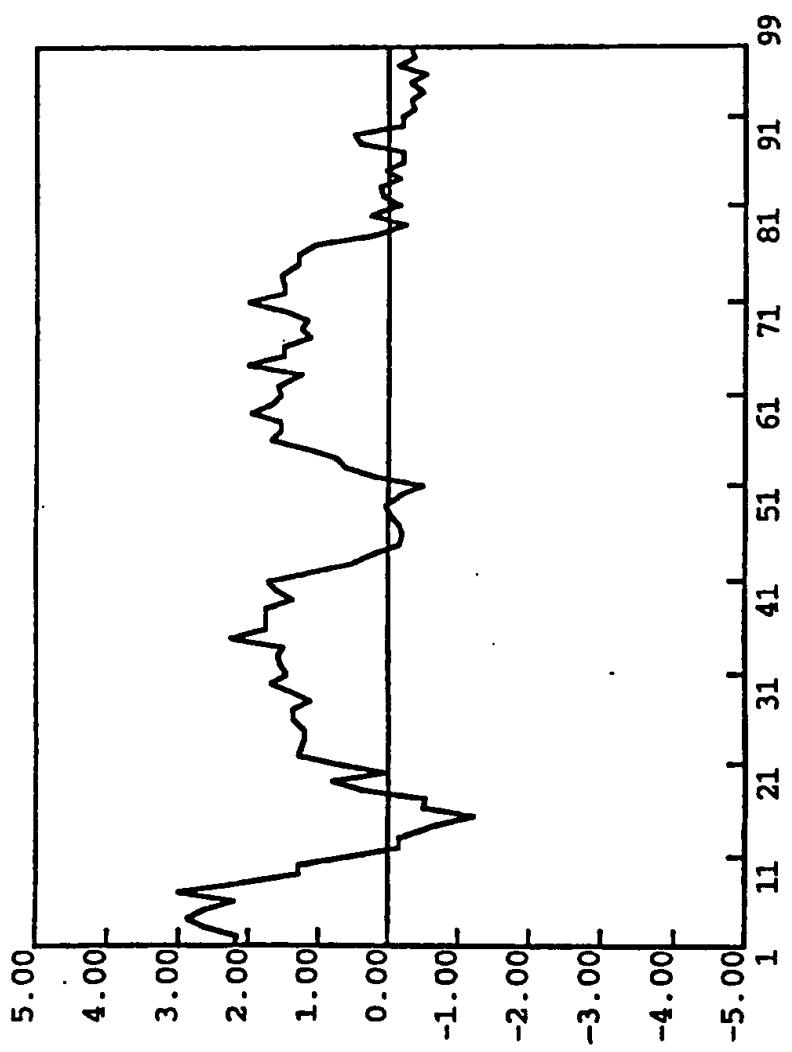
B54	10	20	30	40	50
	GTGGGCAATCGTGGGCAACATCC	TGCTCATATTCTGATCTAACGCTATGC			
RNU04738	X:::	:::	:::	:::	:::
	GTGGGCTCGTAGGAAACGCCCTGGTC	ATTCATATTCTGATCTAACGCTATGC			
	233	243	253	263	273
B54	60	70	80	90	100
	CAAATGAAAGACGCCAACATCTAACCTGCTCAACCTGGCCGTGCTG				
RNU04738	283	293	303	313	323
	CAAATGAAAGACGCCAACATCTAACCTGCTCAACCTGGCCGTGCTG				
	110	120	130	140	150
B54	ATGAGCTCTTCATGCTCAGTGCCATTGCTGGCGGCGTGGCGCTG				
RNU04738	ATGAGCTCTTCATGCTCAGTGCCATTGCTGGCGGCGTGGCGCTG				
	333	343	353	363	373
B54	160	170	180		
	CGCCACTGCGGTTGGGGCGCTGCTGCGC				
RNU04738	CGCCACTGCGGTTGGGGCGCTGCTGCGC			X	
	383	393	403		

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FIGURE 22

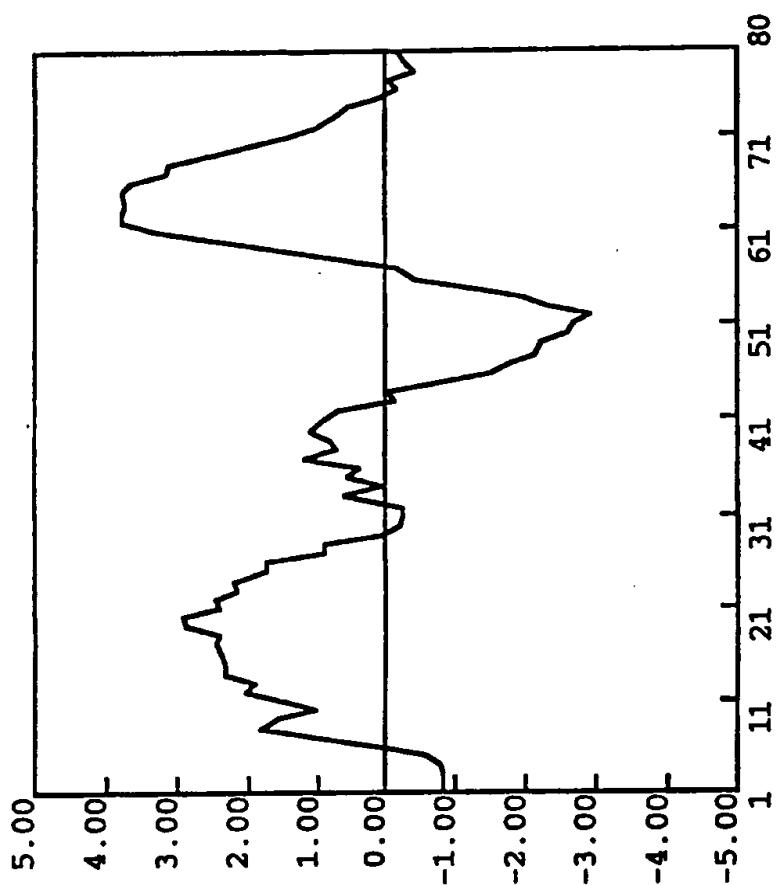
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FIGURE 24



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FIGURE 25



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FIGURE 26

P19P2	1	VGMVGNVL	V	LVIARVRLH	NVTNFLIGNL	AI	SDVLMCTA	CVPILTAYAF	50		
S12863	1	LGVSGNLALI		ILIKQKEMR	NVTNILIVNL	SFS	SDLLVAVM	CLPFTFVYTL	50		
P19P2	51	EPRGWNVF	GG	LICHLVFF	FLOP	VITVYYVS	WFTL	TTHEDRYVG	AGAPAEAGH	100	
S12863	51	MDH-WVFGET		MCKLNPF	VOC	VISITVS	IFSL	VLI	AVERHOL	INPRGWRPN	100
P19P2	101	NRHAYIGITV	I	WVLAVASSL	PFVIVQILTD	EPFQNVSSLAA		FKDKYVCFDK	150		
S12863	101										
P19P2	110		120		130		140		150		
S12863											
P19P2	151	GULLV	Y	LLPL	VIL	LS	---	Y	VRSVKLRNPV	VPVCVTSQA	200
S12863	151	FPSDSHRLSY	T	LLLVLQYF		GPLC	F	ICIFY	FKIYIRLKRR	NNMMDKIRDS	200
P19P2	210		220		230		240		250		
S12863	201	DWDRARRRT	FCL	VVVVVV	F	AICWL	HYY	250	
	201	KYRSSETKRI	NVM	LLSIVVA	F	AVCWL	PLT	250	

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FIGURE 27

9 18 27 36 45 54

5' GTG GCC ATG GTG GGC AAC ATC CTG CTG GTG CTG GTG ATC GCG CCG GTG CGC CGC
Val Gly Met Val Gly Asn Ile Leu Leu Val Ile Ala Arg Val Arg Arg

63 72 81 90 99 108

CTG TAC AAC GTG ACG AAT TTC CTC ATC GGC AAC CTG GCG TTG TCC GAC GTG CTC
Leu Tyr Asn Val Thr Asn Phe Leu Ile Gly Asn Leu Ala Leu Ser Asp Val Leu

117 126 135 144 153 162

ATG TGC ACC GCC TGC GTG CCG CTC ACC CTG GCC TAT GCG TTC GAG CCA CGC GGC
Met Cys Thr Ala Cys Val Pro Leu Thr Leu Ala Tyr Ala Phe Glu Pro Arg Gly

171 180 189 198 207 216

TGG GTG TTC GGC GGC GGC CTG TCC CAC CTG GTC TTC TTC CTG CAG GCG GTC ACC
Trp Val Phe Gly Gly Leu Cys His Leu Val Phe Phe Leu Gln Ala Val Thr

225 234 243 252 261 270

GTC TAT GTG TCG GTG TTC ACG CTC ACC ACC ATC GCA GTG GAC CGC TAC GTC GTG
Val Tyr Val Ser Val Phe Thr Leu Thr Ile Ala Val Asp Arg Tyr Val Val

279 288 297 306 315 324

CTG GTG CAC CCG CTG AGG CCG CGC ATC TCG CTG CGC CTC ACC GCC TAC GCT GTG
Leu Val His Pro Leu Arg Arg Ile Ser Leu Arg Leu Ser Ala Tyr Ala Val

333 342 351 360 369 378

CTG GCC ATC TGG GTG CTG TCC GCG GTG CTG GCG CTG CCC GCC GCC GTG CAC ACC
Leu Ala Ile Trp Val Leu Ser Ala Val Leu Ala Leu Pro Ala Ala Val His Thr

387 396 405 414 423 432

TAT CAC GTG GAG CTC AAG CGG CAC GAC GTG CGC CTC TGC GAG GAG TTC TGG GGC
Tyr His Val Glu Leu Lys Pro His Asp Val Arg Leu Cys Glu Glu Phe Trp Gly

441 450 459 468 477 486

TCC CAG GAG CGC CAG CGC CAG CTC TAC GCC TGG GGG CTG CTG CTG GTC ACC TAC
Ser Gln Glu Arg Gln Leu Tyr Ala Trp Gly Leu Leu Leu Val Thr Tyr

495 504 513 522 531 540

CTG CTC CCT CTG CTG GTG ATC CTC CTG TCT TAC GCC CGG GTG TCA GTG AAG CTC
Leu Leu Pro Leu Leu Val Ile Leu Leu Ser Tyr Ala Arg Val Ser Val Lys Leu

549 558 567 576 585 594

CGC AAC CGC GTG GTG CGG GGC CGC GTG ACC CAG AGC CAG CCC GAC TGG GAC CGC
Arg Asn Arg Val Val Pro Gly Arg Val Thr Gln Ser Gln Ala Asp Trp Asp Arg

603 612 621 630 639 648

GCT CGG CGC CGG CGC ACC TTC TGC TTG CTG GTG GTG GTC GTG GTG GTG TTC ACC
Ala Arg Arg Arg Arg Thr Phe Cys Leu Leu Val Val Val Val Phe Thr

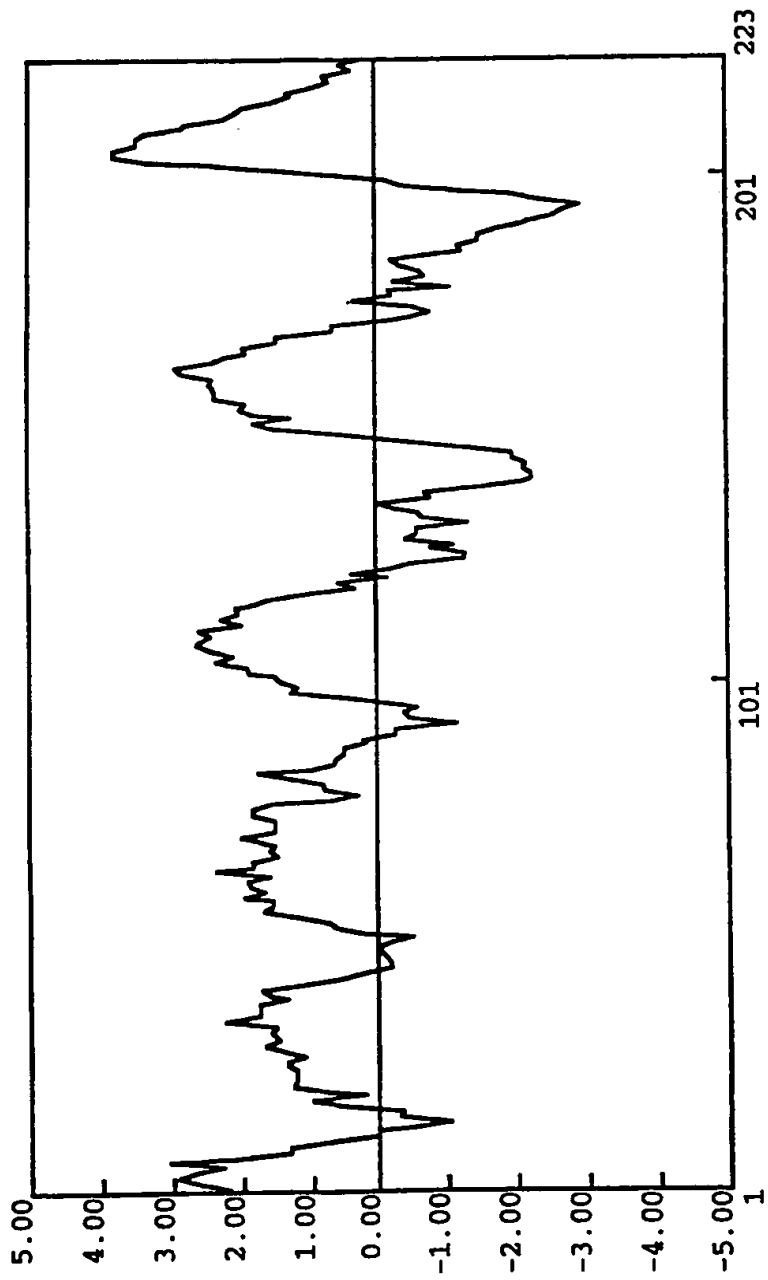
657 666

CTC TCC TGG CTG CCC TTC TIC 3'

Leu Cys Trp Leu Pro Phe Phe

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FIGURE 28



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FIGURE 29

5'	GTC	GCC	ATG	CTG	GGC	AAC	GCC	CTG	GTC	TGT	CAT	GTC	ATC	TTC	AAG	AAC	CAG	CGA	55
	ATG	CAC	TCG	GCC	ACC	ACC	CTC	TTC	ATC	GTC	AAC	CTG	GCA	GTT	GCC	GAC	ATA	ATC	
	Met	His	Ser	Ala	Thr	Ser	Leu	Phe	Ile	Val	Asn	Leu	Ala	Val	Ala	Asp	Ile	Met	
	118	64	73	127	19	28	82	37	91	136	145	154	163						
	ATC	ACG	CTG	CTC	AAC	ACC	CCC	TTC	ACT	TTC	GTT	GCG	TTT	GTC	AAC	AGC	ACA	TGC	
	Ile	Thr	Leu	Leu	Asn	Thr	Pro	Phe	Thr	Leu	Val	Arg	Phe	Val	Asn	Ser	Thr	Trp	
	172	226	181	226	190	235	199	235	208	208	217								
	ATA	TTT	GGG	AAG	GGC	ATG	TGC	CAT	GTC	AGC	CCC	TTT	GCC	CAG	TAC	TGC	TCA	CTG	
	Ile	Phe	Gly	Lys	Gly	Met	Cys	His	Val	Ser	Arg	Phe	Ala	Gln	Tyr	Cys	Ser	Leu	

2 9

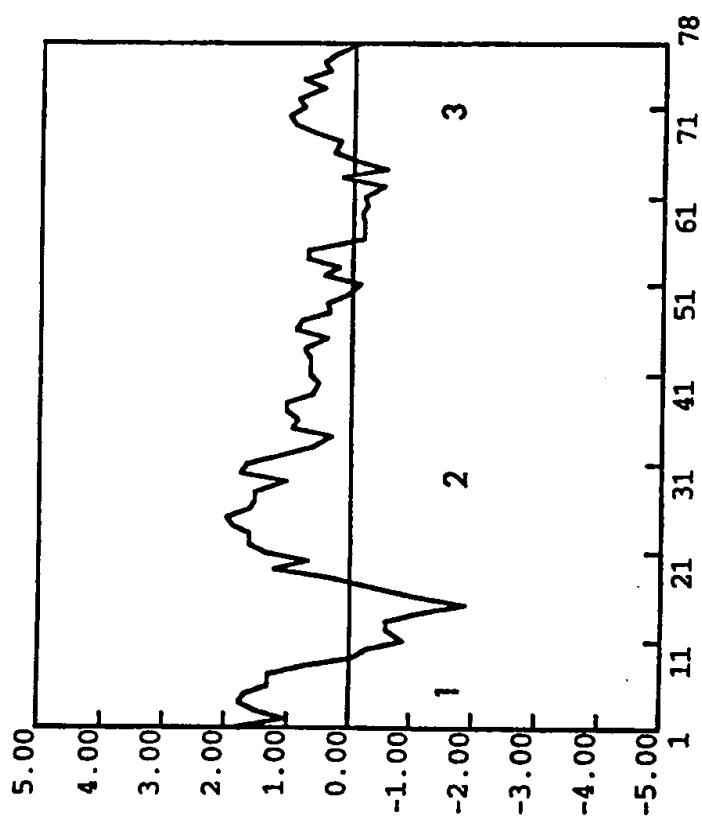
/ 7 9

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FIGURE 30

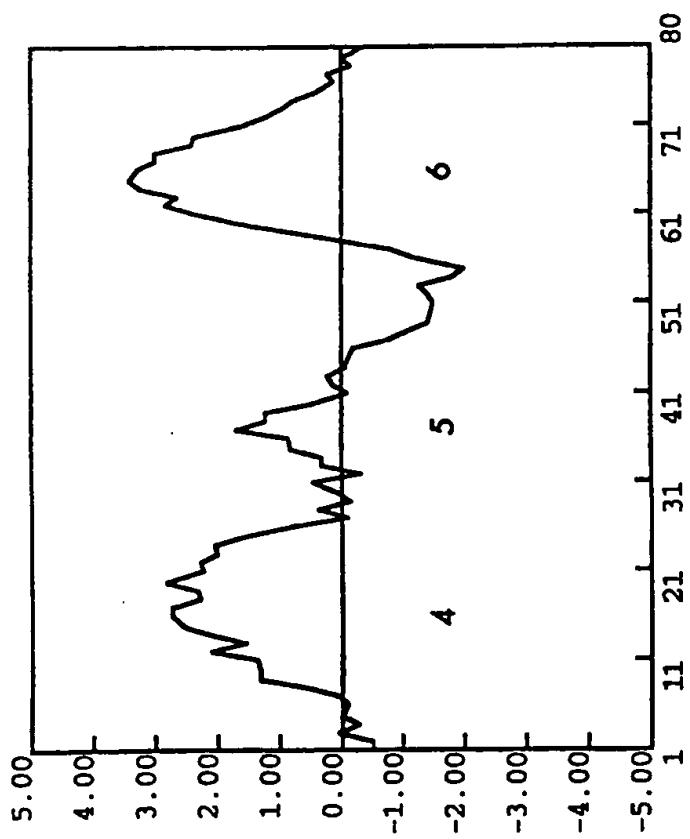
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FIGURE 31



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FIGURE 32



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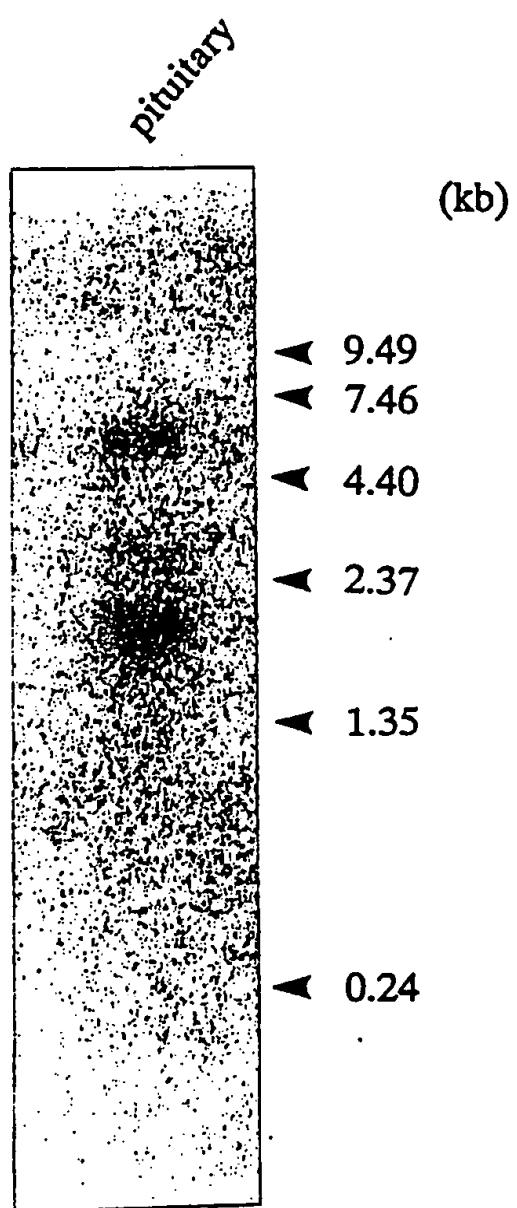
FIGURE 33

	10	20	30	40	50	
p63A2 P30731	1 1	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	50 50
p63A2 P30731	51 51	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	100 100
p63A2 P30731	101 101	[REDACTED] VIWWMATFFS	[REDACTED] LPHAIQCQKLF	[REDACTED] TFKYSEDIVR	[REDACTED] SLCLPDPFP	150 150
p63A2 P30731	151 151	[REDACTED] P	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	200 200
p63A2 P30731	201 201	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	[REDACTED] [REDACTED]	250 250
						...

1	CATCGTCAGCAGATGAACATCATCCACGAGGATGGCTACTCCGAGGCCAGCAGAAATT	60
1		1
61	CTGCCCTCTTCTTCCCCGGAGTCTTCCCGCTCTCCAAACCCCACCTCCAGGTGCCCATG	120
1	Met	1
121	GCGTCATGACCCTCGGGGCCAGGGTTCTGACTTATTTCTGGGCCTGCCGCCGCC	180
1	AlaSerSerThrThrArgGlyProArgValSerAspLeuSerGlyLeuProProAla	21
181	GTCACAACTCCGCCAACCGAGGCCAGGGCTCGCCGCCAACGGCTGGCTGGCTGGC	240
21	ValThrThrProAlaAsnGlnSerAlaGluAlaSerAlaGlyAsnGlySerValAlaGly	41
241	GCGGACGCTCCACCGCTCACGCCCTCCAGGACCTGAGCTTGAGCTGGTGCATCAAGCTGAGGG	300
41	AlaAspAlaProAlaValThrProPheGlnSerIeuGlnLeuValHisGlnLeuIeuGly	61
301	CTGATCGTGTCTCACAGCGTCGGTGGCTGCTGGGCTGGGGCAACTGGCTCTG	360
61	LeuIeuValLeuIeuTyrSerValValValValGlyLeuValGlyAsnCysLeuLeu	81
361	GTGCTGGATCCCGGGCTGGCCCGCTGACACGTGACGAACCTGGCTATGGCAAC	420
81	ValLeuValIleAlaArgValArgArgLeuHisAsnValThrAsnPheLeuIleGlyAsn	101
421	CTGGCCTGTCGACGCTCTCATGTCACCCCTGGCTGCCGCTACGGCTGGCTATGCC	480
101	LeuAlaLeuSerAspValLeuMetCysThrAlaCysValProLeuThrLeuAlaTyrAla	121
481	TTCGACCCACCCCGCTGGCTTCCCGCCCGCCCTGTOCCACCTGGCTCTCTCTGGAG	540
121	PheGluProArgGlyTrpValPheGlyGlyLeuCysHisLeuValPhePheLeuGln	141
541	CGGGTCACCGTCATGTCGGTGTCAACCTCACCAACATCCAGTGGACCGCTACGTC	600
141	ProValThrValTyrValSerValPheThrLeuThrThrIleAlaValAspArgTyrVal	161
601	GTGCTGGTGACCGCTGGGGGGCCATCTGGCTGCCCTCACGCTGTGCTG	660
151	ValLeuValHisProLeuArgArgIleSerLeuArgLeuSerAlaTyrAlaValLeu	181
661	GCCATCTGGGCCCTGCTCCCGTGCTGGGCCCTGCCGCCGCCGCTGCACACCTATCACGTG	720
181	AlaIleTrpAlaIeuSerAlaValLeuAlaLeuProAlaAlaValHisThrTyrHisVal	201
721	GAGCTCAAGCCCCACGACCTGGGCCCTGGGAGGACTCTGGGCTCCAGGAGCCCCAG	780
201	GluLeuIysProHisAspValArgLeuCysGluGluPheTrpGlySerGlnGluArgGln	221
781	CGCCAGCTCTACCGCTGGGCTGCTGGTCACCTACCTGCTCCCTCTGGTCATC	840
221	ArgGlnLeuTyrAlaTrpGlyLeuIeuLeuValThrTyrLeuLeuProLeuLeuValIle	241
841	CTCTCTCTACGCTCCGGCTGTCAGTGAAGCTGGCAACCCGGTGGTGCCGGCTGGCTG	900
241	LeuLeuSerTyrValArgValSerValIleLeuArgAsnArgValValProGlyCysVal	261
901	ACCCAGGCGCAGCCCGACTGGGACCCGCTGGGGGGGGGGGACCTCTGGCTGGCTG	960
261	ThrGlnSerGlnAlaAspTrpAspArgAlaArgArgArgThrPheCysAlaLeuVal	281
961	GTGGCTGGTGGTGTCCCGTCCTGGCTGGGCTGCCAGCTTCACCTGGCTGGCG	1020
281	ValValValValValPheAlaValCysTrpLeuProLeuHisValPheAsnLeuIeu	301
1021	GACCTCGACCCCGACGGCATCGACCCCTAACCCCTTGCGCTGGCTGGCTGAGCACAC	1080
301	AspLeuAspProHisAlaIleAspProTyrAlaPheGlyLeuValGlnLeuLeuCysHis	321
1081	TGGCTGCCATGAGTTGGCTGCTACACCCCTCATCTACGGCTGGCTGCCAGCACAC	1140
321	TrpLeuAlaMetSerSerAlaCysTyrAsnProPheIleItyxAlaTrpLeuHisAspSer	341
1141	TTCCGGAGGACCTGGCAACACTGTTGGCTGGCTGGCCGGCAAGAATAGCCCCCATGCC	1200
341	PheArgGluGluLeuAspArgIysLeuLeuValAlaTrpProArgIysIleAlaProHisGly	361
1201	CAGAAATGACGGTAGCGTGGTCATCTGATGCCACTTAGCCAGGCCCTGGCTAAGGAGC	1260
361	GlnAsnMetThrValSerValValIle***	371
1261	TOCACITCAACTGGCTCCAGGCCACCACTCGAGGTCAATCTGGCTTEATTCTCACCA	1320
371		371
1321	CCAGAGCTAGC	1331
371		371

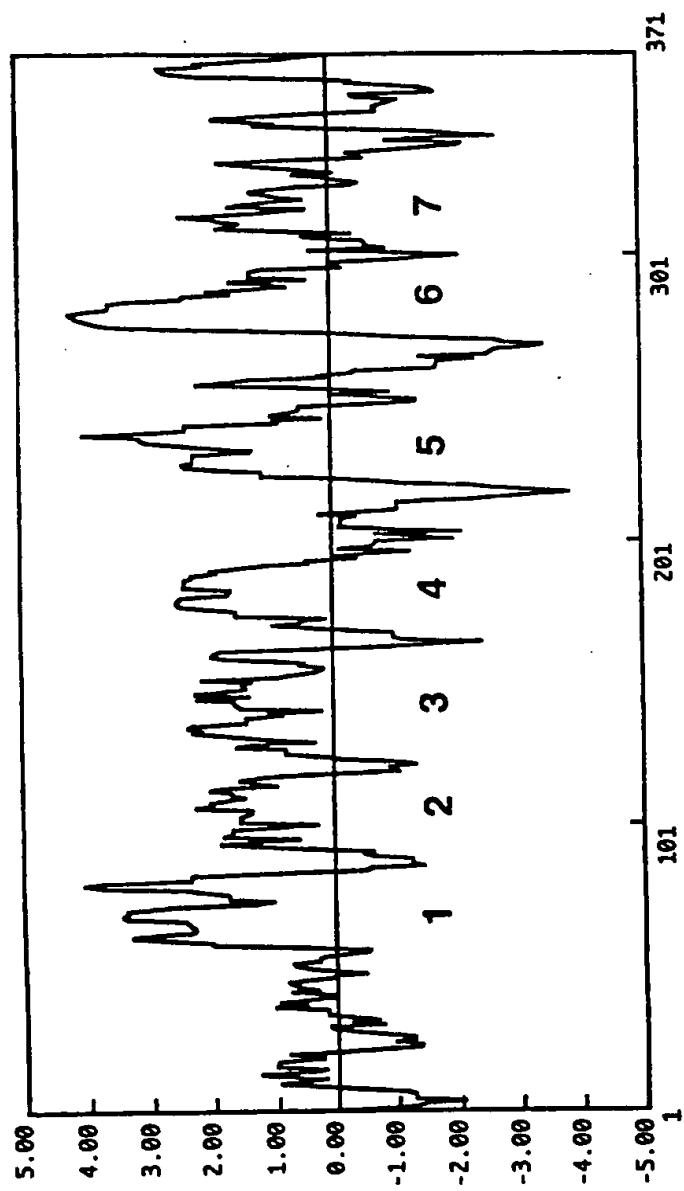
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FIGURE 35



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FIGURE 36



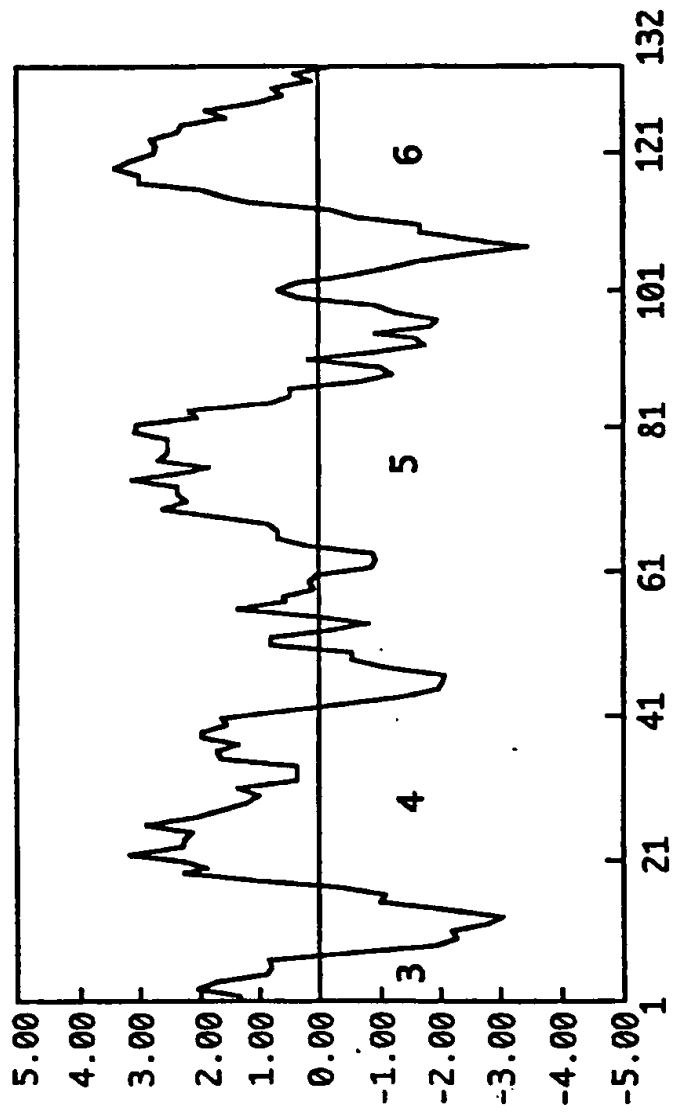
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FIGURE 37

5' 9 18 27 36 45 54
 GTG GGC CTG GTG GGC AAC ATC CTG GCT TCC TGG CAC AAG CGT GGA GGT CGC CGT
Val Gly Leu Val Gly Asn Ile Leu Ala Ser Trp His Lys Arg Gly Gly Arg Arg
 63 72 81 90 99 108
 GCT GCT TGG GCA GTG GTG TGT GCA GTC GTG TGG CTG GCT GTG ACA GCC CAG TGC CTG
Ala Ala Trp Val Val Cys Gly Val Val Trp Leu Ala Val Thr Ala Gln Cys Leu
 117 126 135 144 153 162
 CCC ACG GCA GTC TTT GCT GCC ACA GGC ATC CAG CGC AAC CGC ACT GTG TGC TAC
Pro Thr Ala Val Phe Ala Ala Thr Gly Ile Gln Arg Asn Arg Thr Val Cys Tyr
 171 180 189 198 207 216
 GAC CTG AGC CCA CCC ATC CTG TCT ACT CGC TAC CTG CCC TAT GGT ATG GCC CTC
Asp Leu Ser Pro Pro Ile Leu Ser Thr Arg Tyr Leu Pro Tyr Gly Met Ala Leu
 225 234 243 252 261 270
 ACC GTC ATC GGC TTC TTG CTG CCC TTC ATA GGC TTA CTG CCT TGT TAT TGT CGC
Thr Val Ile Gly Phe Leu Pro Phe Ile Ala Leu Leu Ala Cys Tyr Cys Arg
 279 288 297 306 315 324
 ATG GCC CGC CCC CTG TGT CCC CGG GAT GGC CCA GCA GGT CCT GTG GCC CAA GAG
Met Ala Arg Arg Leu Cys Arg Gln Asp Gly Pro Ala Gly Pro Val Ala Gln Glu
 333 342 351 360 369 378
 CGG CGC AGC AAG GCG GCT CGT ATG GCT GTG GTG GTG GCA GCT GTC TTT GCC CTC
Arg Arg Ser Lys Ala Ala Arg Met Ala Val Val Val Ala Ala Val Phe Ala Leu
 387 396
 TGC TGG CTG CCT CTC TAC 3'
Cys Trp Leu Pro Leu Tyr

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FIGURE 38



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FIGURE 39

		10	20	30	40	50	
P 3H2-17	1	V G I V G N I L A S	W H E G G R R A A	W W V C G V V W L A	V T A Q C I P T A V	F F A A I G Q R N -	5 0
P 3 4 9 9 6	1	R Y T G V H E D K	S I L G E L E K K N A	V Y V S S L U V W A L	V V A V I A P T I L F	Y S G I G V R R N -	5 0
A 4 6 2 2 6	1	R Y L A V V H E T H	S Z R W E T A P V A	R T V S A A V V V A	S A V V V E P V V V	F -- S G V P R E G -	5 0
J N 0 6 0 5	1	R Y V A V V H P L E	A E T Y E R P S V A	K L I N L G V W I A	S L L V T L P I A I	F A D T R P A B G G	5 0
S 2 8 7 8 7	1	R Y T A I P H A T N	S Q K P R K L L A E	K V V Y V G V W L P	A V V L I I P D L I	F A D I K E V D E -	5 0
P 3H2-17	5 1	R I V - C Y L L --	S I P I I S T R Y L	P Y G M A L I V I G	F L L P F I A L L A	C Y C R M A R R I C	1 0 0
P 3 4 9 9 6	5 1	K I I T C Y D T --	T A D E Y L E S Y F	V Y S M C I T V F M	E C I P F I V I L G	C Y G L I V K A I	1 0 0
A 4 6 2 2 6	5 1	M S T - C H M Q V F	E P A A A W H A G E	I I Y -- I I A U L G	E F G P L L V I C I	C Y L L I V V E V E	1 0 0
J N 0 6 0 5	5 1	Q A V A G N L O W P	H P A W S A V F V V	Y T F --- L L G	E L L E P V L A T G I	C Y L L I V G K V E	1 0 0
S 2 8 7 8 7	5 1	R Y I - C D R F --	Y E S D I V L V V E	Q F Q -- H I V V G	L L L P G I V I V S	C Y C I I I S K L S	1 0 0
P 3H2-17	1 0 1	R O D G P -- G E V	A G E - R F S -- k	A A R M A V V V A A	V F A L C W L P L Y	1 5 0
P 3 4 9 9 6	1 0 1	Y K D L D N - S P L	--- R F E -- - k	S I Y L V I I V L T	V F A V S Y L P F H	1 5 0
A 4 6 2 2 6	1 0 1	S A G R E W W A S	C O R R E R S E R R	V I R A V V A V V A	L F V L C W M P F Y	1 5 0
J N 0 6 0 5	1 0 1	A V A L R A -- G	W Q O R R R H S E K K	I I R L V L M V V V	V F V L C W M P F Y	1 5 0
S 2 8 7 8 7	1 0 1	H S K G -- - - -	Y O K R -- - - - k	A L K T T W I L I L	T F F A C W L P Y Y	1 5 0

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FIGURE 40

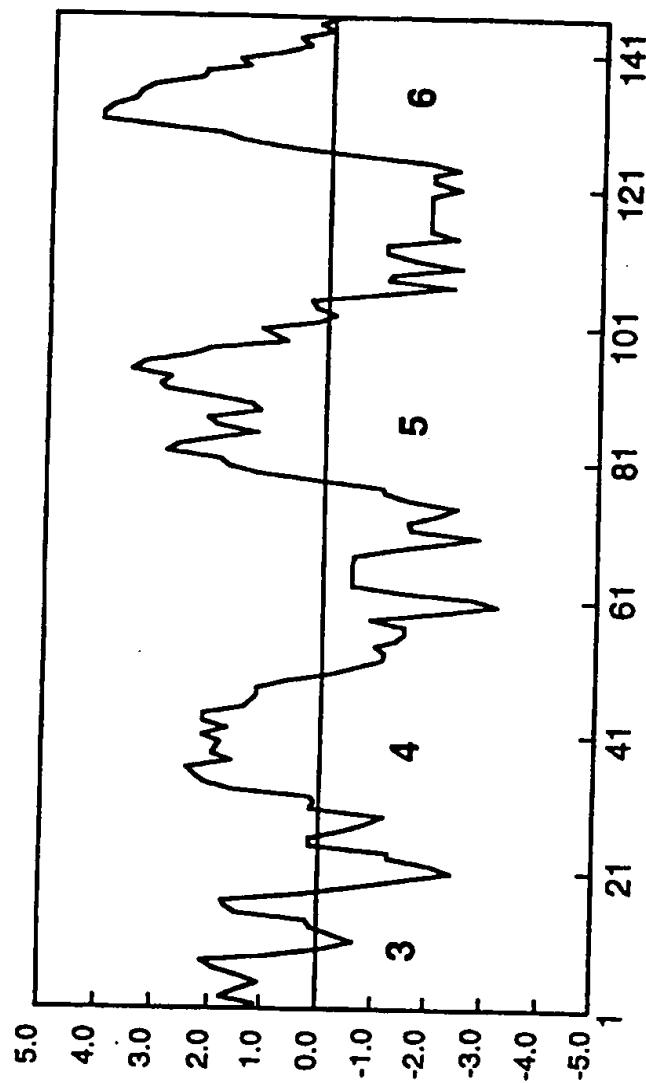
10 19 28 37 46 55
 5' GTG GCC CTG GTG GCC AAC TTC CTG GCC GCG ATG TCT GTG GAT CCC TAC GTG GCC
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Gly Leu Val Gly Asn Phe Leu Ala Ala Met Ser Val Asp Arg Tyr Val Ala
 64 73 82 91 100 109
 ATT GTG CAC TCG CGG CGC TCC TCC TCC CTC AGG GTG TCC CGC AAC GCA CTG CTG
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Val His Ser Arg Arg Ser Ser Leu Arg Val Ser Arg Asn Ala Leu Leu
 118 127 136 145 154 163
 GGC GTG GCC TTC ATC TCG GCG CTG TCC ATC GCC ATG GCC TCG CGG GTG GCC TAC
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Val Gly Phe Ile Trp Ala Leu Ser Ile Ala Met Ala Ser Pro Val Ala Tyr
 172 181 190 199 208 217
 CAC CAG CGT CTT TTC CAT CGG GAC AGC AAC CAG ACC TTC TCC TGG GAG CAG TGG
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Mis Gln Arg Leu Phe His Arg Asp Ser Asn Gln Thr Phe Cys Trp Glu Gln Trp
 226 235 244 253 262 271
 CCC AAC AAG CTC CAC AAC AAG GCT TAC GTG GTG TGC ACT TTC GTC TTT GGG TAC
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Asn Lys Leu His Lys Ala Tyr Val Val Cys Thr Phe Val Phe Gly Tyr
 280 289 298 307 316 325
 CTT CTG CCC TTA CTG CTC ATC TGC TTT TGC TAT GCC AAG GTC CTT AAT CAT CTG
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Leu Pro Leu Leu Ile Cys Phe Cys Tyr Ala Lys Val Leu Asn His Leu
 334 343 352 361 370 379
 CAT AAA AAG CTG AAA AAC ATG TCA AAA AAG TCT GAA GCA TCC AAG AAA AAG ACT
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Mis Lys Lys Leu Lys Asn Met Ser Lys Lys Ser Glu Ala Ser Lys Lys Lys Thr
 388 397 406 415 424 433
 GCA CAG ACC GTC CTG GTG GTC GTT GTC GTC TTT GCC CTC TGC TGG CTG CCT TTC
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Gln Thr Val Leu Val Val Val Val Phe Ala Leu Cys Trp Leu Pro Phe

TAC 3'

Tyr

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FIGURE 41



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FIGURE 42

P3H2-34	1	VOLVGNPLAA	MIVDRYVAA	10	HSFESSSLRV	SFAVLLSEGF	30	40	50
JN0605	1	LSVDRYVAVV	LSVDRYVAVV	10	HELRATVYR	ESVAKLNLG	30	40	50
B41795	1	QETTSIFCUTA	MEDRYVAVV	10	HPIRKAWRE	PEATAKMTMA	30	40	50
A39297	1	QETTSIFCUTA	MEDRYVAVV	10	HPIRKAWRE	FIYAFWVHLG	30	40	50
P3H2-34	51	YV-YNHORLP	HEISNOVTCW	60	70	80	90	100	
JN0605	51	PLAIEFDTIP	AEGDAVACH	51	EENIENKLHK-	-KAYVVCITV	51	51	
B41795	51	PLAIEFDTIP	EDWGRSS-CI	51	LGHFHEAMS-	-AVEVVYTFI	51	51	
A39297	51	PLAIEFDTIP	PLAIEFDTIP	51	INAGSESGA	YTSEFLIIYTFI	51	51	
					MLMFEFRR	LVGFYLITFI			
P3H2-34	101	ET-----	VIANHLKCKK	110	120	130	140	150	
JN0605	101	GICVYI	SKTVALRAGH	101	NMSKSELSK	KTAQAVVLM	101	101	
B41795	101	HECVFHTI	VKSSEGLVGS	101	DPRSESE	SKTHLYLM	101	101	
A39297	101	HECVFHTI	PLCIVIAAP	101	SKRKSE	AKTRHVSII	101	101	
					DKRSESE	RKITLWVMM			
P3H2-34	151	ET-----	-----	160	170	180	190	200	
JN0605	151	ET-----	-----	151	-----	-----	-----	200	
B41795	151	ET-----	-----	151	-----	-----	-----	200	
A39297	151	ET-----	-----	151	-----	-----	-----	200	

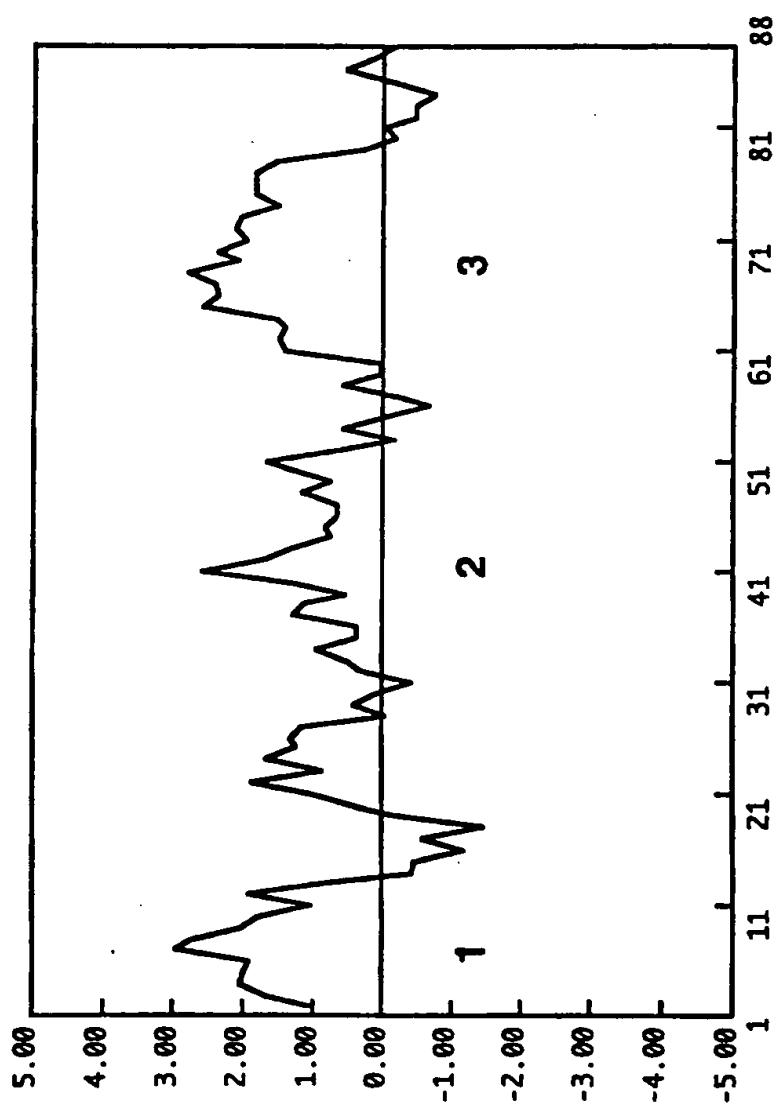
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FIGURE 43

5'	GTC	GCC	ATG	GTC	GCC	AAC	GTC	CAG	GTC	CTC	TGG	TTC	TTC	GCC	TTC	TCC	TTC	ATC	AAG	55
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Val	Gly	Met	Val	Gly	Asn	Val	Leu	Val	Leu	Tyr	Phe	Phe	Phe	Gly	Ser	Ile	Phe	Ser	Lys	
64	64	73	82	91	91	100	109													
AGG	ACC	CCC	TTC	GTC	TAC	TTC	CTG	CAC	CTG	GCC	AGC	GCC	GAC	GCC	GCC	GCC	GCC	TAC		
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
Arg	Thr	Pro	Phe	Ser	Val	Tyr	Phe	Leu	His	Leu	Ala	Ser	Ala	Asp	Gly	Gly	Ala	Tyr		
118	118	127	136	145	145	154	163													
CTC	TTC	AGC	AAG	GCC	GTG	TTC	TCC	CTG	AAC	GCC	GGC	GCC	TTC	CTG	GCC	GCC	ACC			
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
Leu	Phe	Ser	Lys	Ala	Val	Phe	Ser	Leu	Leu	Asn	Ala	Gly	Gly	Phe	Leu	Gly	Tyr			
172	172	181	190	199	199	208	217													
TTC	GCC	CAC	TAT	GTG	CCC	ACC	GTG	GCC	CGG	GTG	CTG	GGG	CTG	GCC	TTC	TTC	GTG			
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
Phe	Ala	His	Tyr	Val	Arg	Ser	Val	Ala	Arg	Val	Leu	Gly	Leu	Cys	Ala	Phe	Val			
226	226	235	244	253	253	262														
GCG	GCC	GTG	AGC	CTC	CTG	CCG	GCC	GTG	AGC	ATG	GA	GCG	TGC	GCG	TCT	G	3'			
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
Ala	Gly	Val	Ser	Leu	Leu	Pro	Ala	Val	Ser	Met	Glu	Arg	Cys	Ala	Ser					

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FIGURE 44



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FIGURE 45

pMD4	1	VEMVGVVLVI	10	WFFGFSIKRTI	20	PFFSIVYFLHLA	30	SADCVYLSK	40	AVFSLLNAGG	50	50
A35639	1	CSLIGNGLVI	1	WFFGFSIKRTI	1	PFFSIVYFLHLA		SADCVYLSK		AVIALIMGR		
pMD4	51	FLCLPFAHIVF	60	SIAAEVILGLCA	70	FVAGVSLLPA	80	VSMERCAAS..	90	100	100	100
A35639	51	FLGSIEDIVF		RVSRSIVGLCP		FVAGVSLLPA		IHEVCYVS..				

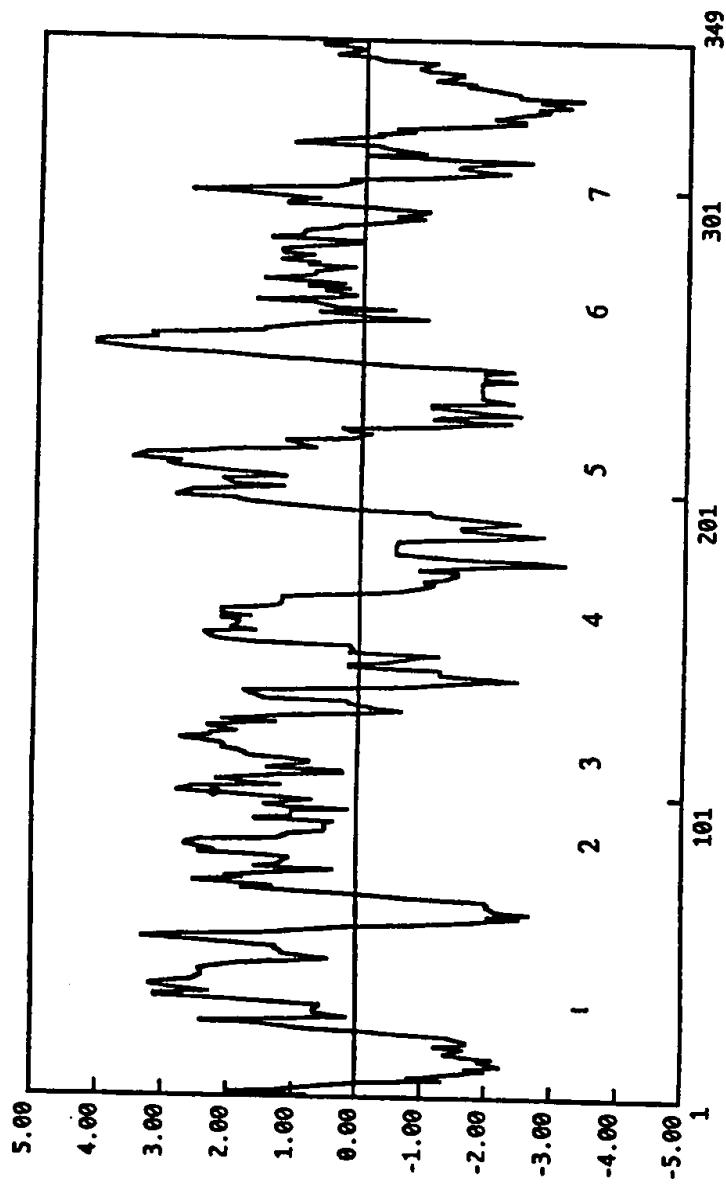
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FIGURE 46

1	CAAAGCAACACGGTCAACCTCAAGGACTGAAAGCAACGGGACCCAGCTCACAAAGGCCAACGGATTGAACC	72
1		1
73	CATAACCGCTCAGAAGATTTCTCGGCGCTGGGAGAGCTCGGAGGAGTCCTCACCCGTCAGCTTGACTGC	144
1		1
145	GAGCACTGAGAGTCGCGTAGACCCGTAACCTCTGTGTCCTGGAGGCTGCCGCCCCCGACGGAAAGGCTTAG	216
1		1
217	CTCGGGACTTGCAGCACCCCTCTTACGGCAGGCCAGGGAGGATAGTGTGATCGGCACAGCCAGG	288
1		1
289	GTGGCTCTTCAGGCTTCTTGCGGGTTGCGGGAGGTACTAGTTGGAGACGGCGGCCCTGGCTCTGGCGCT	360
1		1
361	CTGTCTGGGCCACTCCGTGATCCTAGGCTACCTCCAGAGCCAGTTTCCCTGGCTGGCACAACTCTCCAGG	432
1		1
433	GCGCTCCGGTCCGGTACAGCGCCCCAAGGGGTATCCCAGTAAGTGAAGGAACTGGCTATGGTGAACCTC	504
1	MetGluleuAlaMetValAsnLeu	8
505	AGTGAAGGAAATGGGAGCCACAGAGCCCCAGCCCCGAGTCCAGGGCGCTTTCGGCATGGCTGGAG	576
8	SerGluGlyAsnGlySerAspProGluProProAlaProGluSerArgProLeuPheGlyIleGlyValGlu	32
577	AACTICATTACCTGGTACTGTTTGGCTGATTTCCGGATGGGGCTGCTGGGCAACNGGCTGGTGAATCACC	648
32	AsnPheIleThrLeuValValPheGlyLeuIlePheAlaMetGlyValLeuGlyAsnSerLeuValIleThr	56
649	GTGCTGGCCGCGCAGCAAACCAAGGCAAGCCGCCAGCACCAACCTGTTATCCTCAATCTGAGCATCGCA	720
56	ValLeuAlaArgSerLysProGlyLysProArgSerThrThrAsnLeuPheIleLeuAsnLeuSerIleAla	80
721	GACCTGGCCTACCTGCTCTCTGCATCCCTTTCAGGCCACCGTGTATGCACTGCCAACCTGGTGCTGGC	792
80	AspLeuAlaTyrLeuLeuPheCysIleProPheGlnAlaThrValTyrAlaLeuProThrTrpValLeuGly	104
793	GCCTTCATCTGCAAGTTTACACTACTCTTCACCGTGTCCATGCTGGAGCATTCACCCCTGGCCGOG	864
104	AlaPheIleCysLysPheIleHistYxPhePheThrValSerMetLeuValSerIlePheThrLeuAlaAla	128
865	ATGTCTGTGGATCGCTACGTGGCCATTGTCACCTGGGGCGCTCCCTCCCTCAGGGTGCTCCGCAACGCA	936
128	MetSerValAspArgIlyrValAlaIleValHisSerArgSerSerLeuArgValSerArgAsnAla	152
937	CTGCTGGGCGTGGCTTCATCTGGCGCTGCCATGCCATGGCTGCCGTCGGCTACACCACAGGGCTTT	1008
152	LeuLeuGlyValGlyPheIleTrpAlaLeuSerIleAlaMetAlaSerProValAlaTyrHisGlnArgLeu	176
1009	TTCATCGGGACGCAACCAAGACCTCTGCTGGGACGCTGGCCAACAAAGCTCCACAAAGAAGGCTTACGTG	1080
176	PheHisArgAspSerAsnGlnThrPheCysTrpGluGlnTrpProAsnLysLeuHisLysAlaTyrVal	200
1081	GTTGCACTTCTGCTTGGGTACCTCTGCCCTTACTGCTCATCTGCTTTGCTATGCCAACGGTCTTAAAT	1152
200	ValCysThrPheValPheGlyTyrLeuLeuProLeuLeuIleCysPheCysTyzAlaLysValLeuAsn	224
1153	CATCTGCATAAAAAGCTAAAAACATGTCAAAAAAGCTGAACCATCAGAAAAAGACTGCACAGACCGTC	1224
224	HisLeuHisLysLeuLysAsnMetSerLysLysSerGluAlaSerLysLysThrAlaGlnThrVal	248
1225	CTGGCTGGTCTGTGAGTATTGGCATATCTGGCTGCCCATCATGTCGTCACCCCTGGCTGAGTTGGAA	1296
248	LeuValValValValPheGlyIleSerTrpLeuProHisHisValValHisLeuTrpAlaGluPheGly	272
1297	GCCTTCCCACATGAGGCCAGCTCCCTCTCTCAGAACATACGGCCCATGCGCATACAGCAACTCTCA	1368
272	AlaPheProLeuThrProAlaSerPhePhePheArgIleThrAlaHisCysLeuIleTyrSerAsnSer	296
1369	GTGAACCCCATCATATGCCCTCTCTCAGAAAAACTCCGGAGGGGTACAAGCAAGTGTCAAGTGTCA	1440
296	ValAsnProIleIleTyrAlaPheLeuSerGluAsnPheArgLysAlaTyrLysGlnValPheLysCysHis	320
1441	GTTGCGATGAATCTCACCGCAGTGAAACTAAGGAAACAAAGAGCCGGATGGACACCCCGCATCCACCAAC	1512
320	ValCysAspGluSerProArgSerGluThrLysGluAsnLysSerArgMetAspThrProProSerThrAsn	344
1513	TGCACCCACCTGTGAAGGTTGGGGAGCCCTCCGACTTCCAGCTCCCATGIGCTTAGAGAGAGGGAGGGCG	1584
344	CysThrHisVal***	349
1585	GAGCGAATTATCAAGTAACATGG	1607
349		349

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FIGURE 47



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FIGURE 48

MOUSEGALRECE HUNGALAMI	1	SELMVNLSE	10	ENGSDPEPPE	20	PESRPILEGIC	30	VENPFLAVWV	40	SHIFAMGVIG	50
MOUSEGALRECE HUNGALAMI	51	ISLYTIVLAE	60	SKFGCKPRSTI	70	SKFGCKPRSTI	80	DLFILMLSTIA	90	FOAIVYALEP	100
MOUSEGALRECE HUNGALAMI	101	MLGAF1CKF	110	IHFIFTIVSEL	120	ISIFULAAHS	130	IDRYVAVIHS	140	MRSSSSLRVSI	150
MOUSEGALRECE HUNGALAMI	151	MLGAF1CKF	160	IHFIFTIVSEL	170	AYHCFSLFHP	180	DSSQFCNEC	190	IPPNLAKKAY	200
MOUSEGALRECE HUNGALAMI	201	VALGWVFH	210	ALSIASASPV	220	AKVLNLHKKI	230	AKNESSHSEA	240	SKERETAQIVI	250
MOUSEGALRECE HUNGALAMI	251	AVVVFVFGYI	260	IPHAIVVHLWV	270	EFCAFPLTPA	280	SFERITPAIC	290	AYSVNSSVNI	300
MOUSEGALRECE HUNGALAMI	301	IVYAFLSIEH	310	AKAYKQVFKC	320	TCODEFSRSE	330	TKENKSRIID	340	EPSTRUCTIVK	350
MOUSEGALRECE HUNGALAMI	351	X.....	360	AKAYKQVFKC	370	TKRKSLSID	380	TKENKSRIID	390	EPSTRUCTIVK	400
										400

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FIGURE 49

5' CTC GCG GCT CTG GGT ATG GAT CGG TAT CTT CTC ACC CTT CAC CCA GTG TGG TCC
 9 18 27 36 45 54
 Leu Leu Thr Leu His Pro Val Trp Ser

CAA AAG CAC CGA ACC TCA CAC TGG GCT TCC AGA GTC GTT CTG GGA GTC TGG CTC
 63 72 81 90 99 108
 Gln Lys His Arg Thr Ser His Trp Ala Ser Arg Val Val Leu Gly Val Trp Leu

TCT GCC ACT GCC TTC AGC GTG CCC TAT TTG GTT TTC AGG GAG ACA TAT GAT GAC
 117 126 135 144 153 162
 Ser Ala Thr Ala Phe Ser Val Pro Tyr Leu Val Phe Arg Glu Thr Tyr Asp Asp

CGT AAA GGA AGA GTG ACC TGC AGA AAT AAC TAC GCT GTG TCC ACT GAC TGG GAA
 171 180 189 198 207 216
 Arg Lys Gly Arg Val Thr Cys Arg Asn Asn Tyr Ala Val Ser Thr Asp Trp Glu

AGC AAA GAG ATG CAA ACA GTA AGA CAA TGG ATT CAT GCC ACC TGT TTC ATC AGC
 225 234 243 252 261 270
 Ser Lys Glu Met Gln Thr Val Arg Gln Trp Ile His Ala Thr Cys Phe Ile Ser

CGC TTC ATA CTG GGC TTC CTT CTG CCT TTC TTA GTC ATT GCC TTT TGT TAT GAA
 279 288 297 306 315 324
 Arg Phe Ile Leu Gly Phe Leu Leu Pro Phe Leu Val Ile Gly Phe Cys Tyr Glu

AGA GTA GCC CGC AAG ATG AAA GAG AGG GGC CTC TTT AAA TCC AGC AAA CCC TTC
 333 342 351 360 369 378
 Arg Val Ala Arg Lys Met Lys Glu Arg Gly Leu Phe Lys Ser Ser Lys Pro Phe

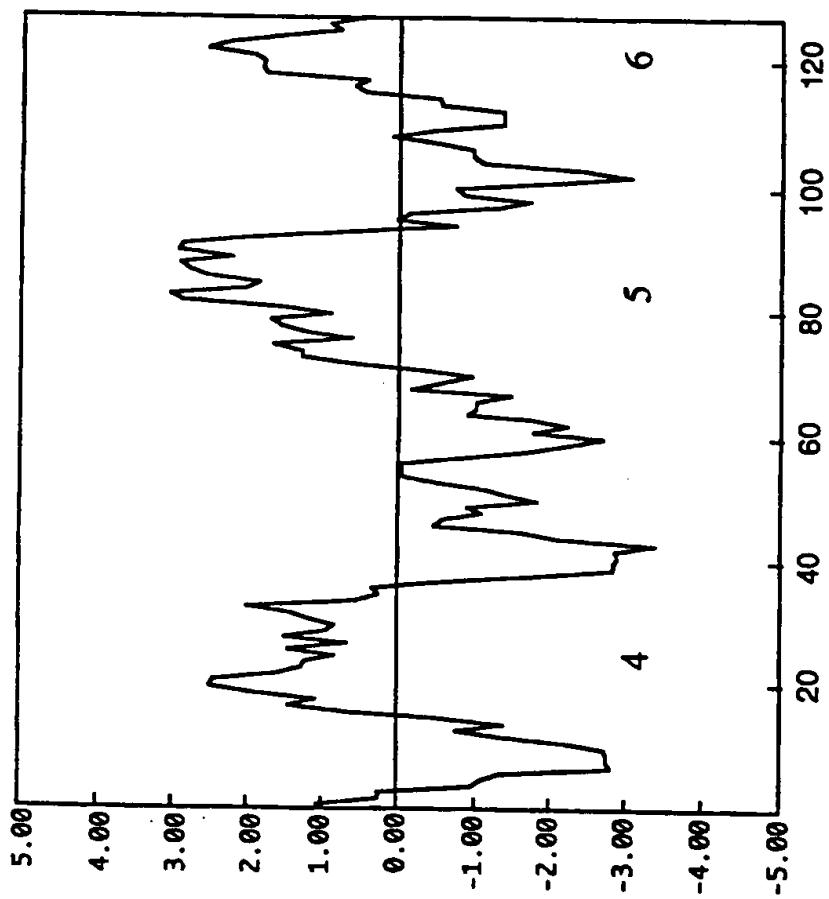
AAA GTC AGC ATG ACT GCT GTT ATC TCT TTT TTC TGT CCT GGC TTC CCT ACC ACA
 387 396 405 414 423 432
 Lys Val Thr Met Thr Ala Val Ile

TG 3'

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FIGURE 50



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FIGURE 51

PMJ10		10	KIETSHMASR	MLVLSAT	AFSVEXLVE	EYDOR-K-C	50
B42009	1	TCVHLHPTAC	THFTVSLAKE	VITLGPETIL	VITLGPETIL	TIVTIE-N-C	50
JC2014	1	VCVHLHPTAC	THFTVSLAKE	VITLGPVMAI	VITLGPVMAI	TIVEGK-T-	50
A46520	1	ICVHLHPTAC	THFTVSLAKE	VITLGPICAL	VITLGPICAL	TILSHTRAP	50
A46525	1	LIEKELTAC	KVFGTGLAW	ACGVAWIAI	ELLTIESFVYE	EAXEDEFS-E	50
S28787	1	ATVAVTNS	KPKTLLAEV	IVYVNLPV	ELTFEDLIA	DI-EV-D-E	50
		20					
		30					
		40					
		50					
		60					
		70					
		80					
		90					
		100					
PMJ10	51	ATENNYV	SIBESKEO	TDRWTATC	EISREFLSE	IPFLVIGFY	100
B42009	51	DYCTTENS	GG-TEERI	KVAAIT	SHIREVIGFS	LPHSIVAVCY	100
JC2014	51	TVACTTENS	WIN-DEKEET	KVAAIT	SHIRFLIGFS	APHSIVAVSY	100
A46520	51	XMACTTDS	WE-DEAKI	KVAAIS	SHIRFLIGFS	TPHSIVAVCY	100
A46525	51	HVEGLIG	GS-FEKKA	VA-	-118MVEV	HPHTNICY	100
S28787	51	XTDRFP-	SDLI	-	--IVVCEV	EGIVILSCY	100
		110					
		120					
		130					
		140					
		150					
PMJ10	101	EVETRACE	GIEKSEEFK	VIETTAI	
B42009	101	ELIAKTHIK	GMIKSSRPLF	VIITVW	150
JC2014	101	ELIAKTHIQ	GLIKSSRPLF	VIISFW	150
A46520	101	ELIAKTHRO	GLIKSSRPLF	VIISFW	150
A46525	101	TFILLRTWS	KATR EIKT	VIAVWII	150
S28787	101	CIIKSHS	KGXQKRKA	TTVILLI	150

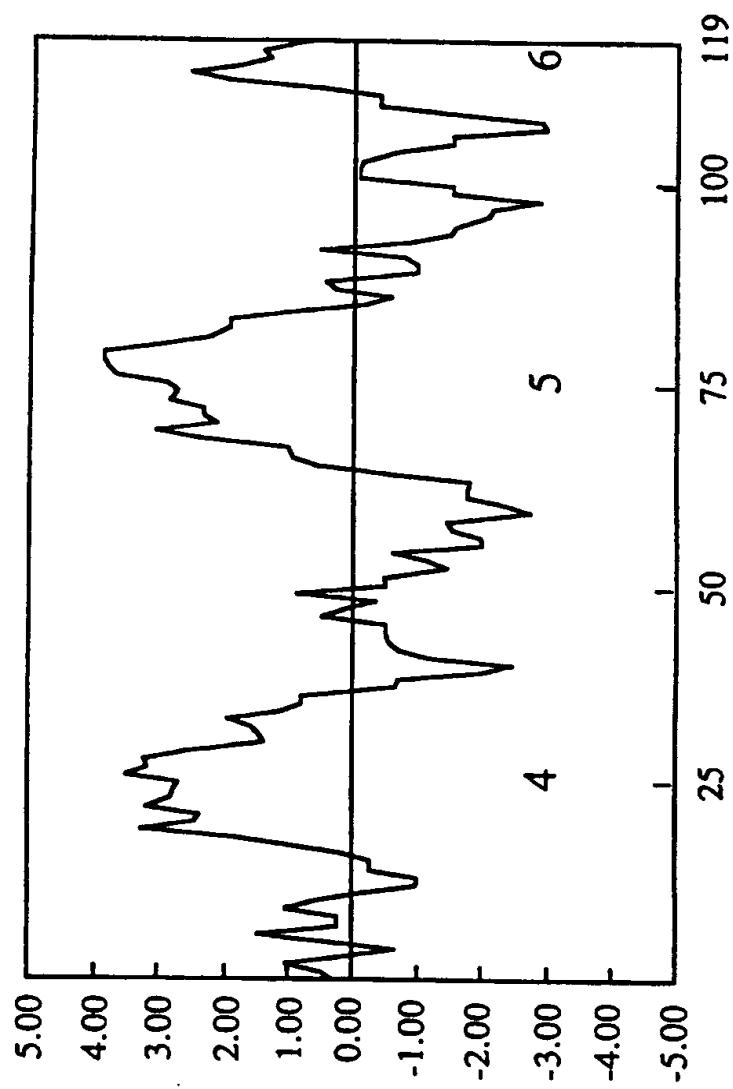
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FIGURE 52

9 18 27 36 45 54
 CTG ACT GCT CTG GGG ACT GAC CGG TAT TTC AAG ATT GTG AAG CCC CTT TCC ACG
 --- --- --- --- --- ---
 Phe Lys Ile Val Lys Pro Leu Ser Thr
 63 72 81 90 99 108
 TCC TTC ATC CAG TCT GTG AAC TAC AGC AAA CTC GTC TCG CTG GTG GTC TGG TTG
 --- --- --- --- --- ---
 Ser Phe Ile Gln Ser Val Asn Tyr Ser Lys Leu Val Ser Leu Val Val Trp Leu
 117 126 135 144 153 162
 CTC ATG CTC CTC CTC GCC GTC CCC AAC GTC ATT CTC ACC AAC CAG AGA GTT AAG
 --- --- --- --- --- ---
 Leu Met Leu Leu Ala Val Pro Asn Val Ile Leu Thr Asn Gln Arg Val Lys
 171 180 189 198 207 216
 GAC GTG ACG CAG ATA AAA TGC ATG GAA CTT AAA AAC GAA CTG GCC CGC CAG TGG
 --- --- --- --- --- ---
 Asp Val Thr Gln Ile Lys Cys Met Glu Leu Lys Asn Glu Leu Gly Arg Gln Trp
 225 234 243 252 261 270
 CAC AAG GCG TCA AAC TAC ATC TTT GTG GGC ATT TTC TGG CTT GTG TTC CTT TTG
 --- --- --- --- --- ---
 His Lys Ala Ser Asn Tyr Ile Phe Val Gly Ile Phe Trp Leu Val Phe Leu Leu
 279 288 297 306 315 324
 CTA ATC ATT TTC TAC ACT GCT ATC ACC AGG AAA ATC TTT AAG TCC CAC CTG AAA
 --- --- --- --- --- ---
 Leu Ile Ile Phe Tyr Thr Ala Ile Thr Arg Lys Ile Phe Lys Ser His Leu Lys
 333 342 351 360 369 378
 TCC AGA AAG AAT TCC ATC TCG GTC AAA AAG AAA TCT AGC CGC AAC ATC TTC AGC
 --- --- --- --- --- ---
 Ser Arg Lys Asn Ser Ile Ser Val Lys Lys Ser Ser Arg Asn Ile Phe Ser
 387 396 405 414
 ATC GTG TTT ATC CTC TGT TGG CCC CCC TAC CAC ATC 3' .
 --- --- --- --- ---
 Ile Val

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FIGURE 53



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FIGURE 54

DMH28	1	FKIVKPLSIS	FQSVNYSKL	VSLVWILLM	LLAVENVII	NOVKDVTQI	50
P35343	1	LAVVHATSP-	LICKRHLWKF	YCIAMMLSV	ILALPILIR	SPVKVNLSL	50
A41795	1	PAVVFHKAA	RXBRFIVAKV	VNLGVIVEST	LVTLPVVES	RFLANSDCIV	50
A47457	1	PAVVFIRAA	TYRRESVAKL	TNLGVMLASL	LVTLEAIFA	DIRPARGCQ-	50
DMH28	51	KCME-EKAEI	GFOGRKASNY	[FVG1 F-WI]	FELLIFYTA	IT-RMIFRSI	100
P35343	51	VCYEDVGET	SEL--RVUUR	LPQTEGEIV	PLTMILECYG	FTLRTLFKAH	100
A41795	51	ACNM-EPEE	AQRGLUGEV-	LYTFPLGFL	FGCATICLCYV	LILAKMRVVA	100
A47457	51	AVAC-NLQW	HPSA2EV-	VITFL3FL	EMLAISCLYL	LIVGKHEVA	100
DMH28	101	LSRKNSI-S	VKKISSNIF	S--IV	130	140	150
P35343	101	MG---EKRH	AMR-----VIF	AVVIV	150
A41795	101	ERACHCOKE	SERKITIAVM	SVVIV	150
A47457	101	ERAGCQORH	SERKITEVL	SVVIV	150

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FIGURE 55

9 18 27 36 45 54

5' GCC ACC AAC GTG TTC ATC CTG TGT CTG GTG GAC CTG CTG GCT GCC CTG ACC CTC

Val Asp Leu Leu Ala Ala Leu Thr Leu

63 72 81 90 99 108

ATG CCT CTG GCC ATG CTC TCC AGC TCC GCC CTC TTT GAC CAC GCC CTC TTT GGG

Met Pro Leu Ala Met Leu Ser Ser Ala Leu Phe Asp His Ala Leu Phe Gly

117 126 135 144 153 162

GAG GTG GCC TGC CGC CTC TAC TTG TTC CTG AGC GTC TGC TTT GTC AGC CTG GCC

Glu Val Ala Cys Arg Leu Tyr Leu Ser Val Cys Phe Val Ser Leu Ala

171 180 189 198 207 216

ATC CTC TCG GTG TCC GCC ATC AAT GTG GAG CGC TAC TAT TAT GTG GTC CAC CCC

Ile Leu Ser Val Ser Ala Ile Asn Val Glu Arg Tyr Tyr Val Val His Pro

225 234 243 252 261 270

ATG CGC TAT GAG GTG CGC ATG AAA CTG GGG CTG GTG GCC TCT GTG CTG GTG GGC

Met Arg Tyr Glu Val Arg Met Lys Leu Gly Leu Val Ala Ser Val Leu Val Gly

279 288 297 306 315 324

GTG TGG GTG AAG GCC CTG GCC ATG GCT TCT GTG CCA GTG TTG GGA AGG GTG TCC

Val Trp Val Lys Ala Leu Ala Met Ala Ser Val Pro Val Leu Gly Arg Val Ser

333 342 351 360 369 378

TGG GAG GAA GGC CCT CCC AGT GTC CCC CCA GGC TGT TCA CTC CAA TGG AGC CAC

Trp Glu Glu Gly Pro Pro Ser Val Pro Pro Gly Cys Ser Leu Gln Trp Ser His

387 396 405 414 423 432

AGT GCC TAC TGC CAG CTT TTC GTG GTG GTC TTC GCC GTC CTC TAC TTC CTG CTG

Ser Ala Tyr Cys Gln Leu Phe Val Val Phe Ala Val Leu Tyr Phe Leu Leu

441 450 459 468 477 486

CCC CTG CTC CTC ATC CTT GTG GTC TAC TGC AGC ATG TTC CGG GTG GCT CGT GTG

Pro Leu Leu Leu Ile Leu Val Val Tyr Cys Ser Met Phe Arg Val Ala Arg Val

495 504 513 522 531 540

GCT GCC ATG CAG CAC GGG CCG CTG CCC ACG TGG ATG GAG ACC CGG CCC CGG CAA CGC

Ala Ala Met Gln His Gly Pro Leu Pro Thr Trp Met Glu Thr Pro Arg Gln Arg

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FIGURE 56

549 558 567 576 585 594
TCC GAG TCT CTC AGC AGC CGC TCC ACT ATG GTC ACC AGC TCG GGG GCC CCG CAG
--- --- --- --- --- ---
Ser Glu Ser Leu Ser Ser Arg Ser Thr Met Val Thr Ser Ser Gly Ala Pro Gln

603 612 621 630 639 648
ACC ACC CCT CAC CGG ACG TTT GGC GGA GGG AAG GCA GCA GTG GTC CTC CTG GCT
--- --- --- --- --- ---
Thr Thr Pro His Arg Thr Phe Gly Gly Lys Ala Ala Val Val Leu Leu Ala

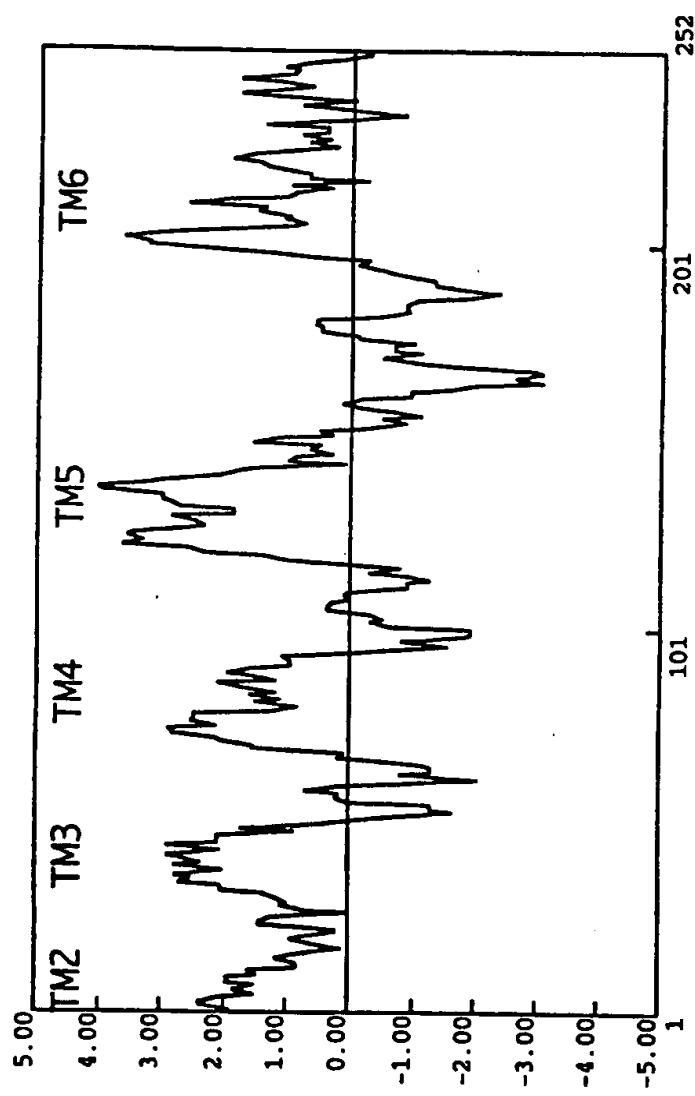
657 666 675 684 693 702
GTG GGA GGA CAG TTC CTG CTC TGT TGG CCC TAC TTC TCC TTC CAC CTC TAT
--- --- --- --- --- ---
Val Gly Gly Gln Phe Leu Leu Cys Trp Leu Pro Tyr Phe Ser Phe His Leu Tyr

711 720 729 738 747 756
GTG GCC CTG AGC GCT CAG CCC ATT GCA GCG GGG CAG GTG GAG AAC GTG GTG ACC
--- --- --- --- --- ---
Val Ala Leu Ser Ala Gln Pro Ile Ala Ala Gly Gln Val Glu Asn Val Val Thr

765 774 783 792 801 810
TGG ATT GGC TAC TTC TGC TTC ACC TCC AAC CCT CTC CTC TAT TCC TTC CTC CCT 3'
--- --- --- --- --- ---
Trp Ile Gly Tyr Phe Cys Phe Thr Ser

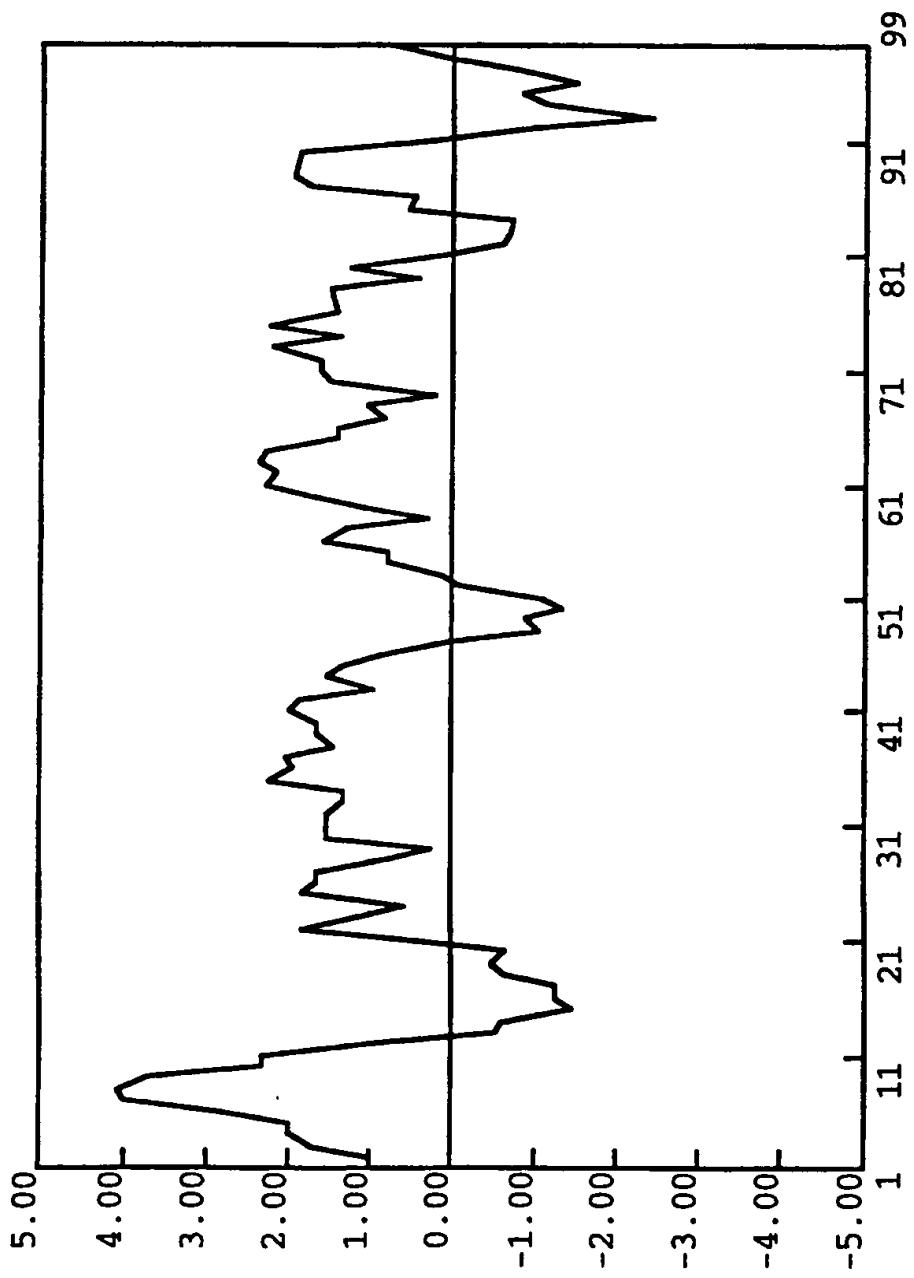
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FIGURE 57



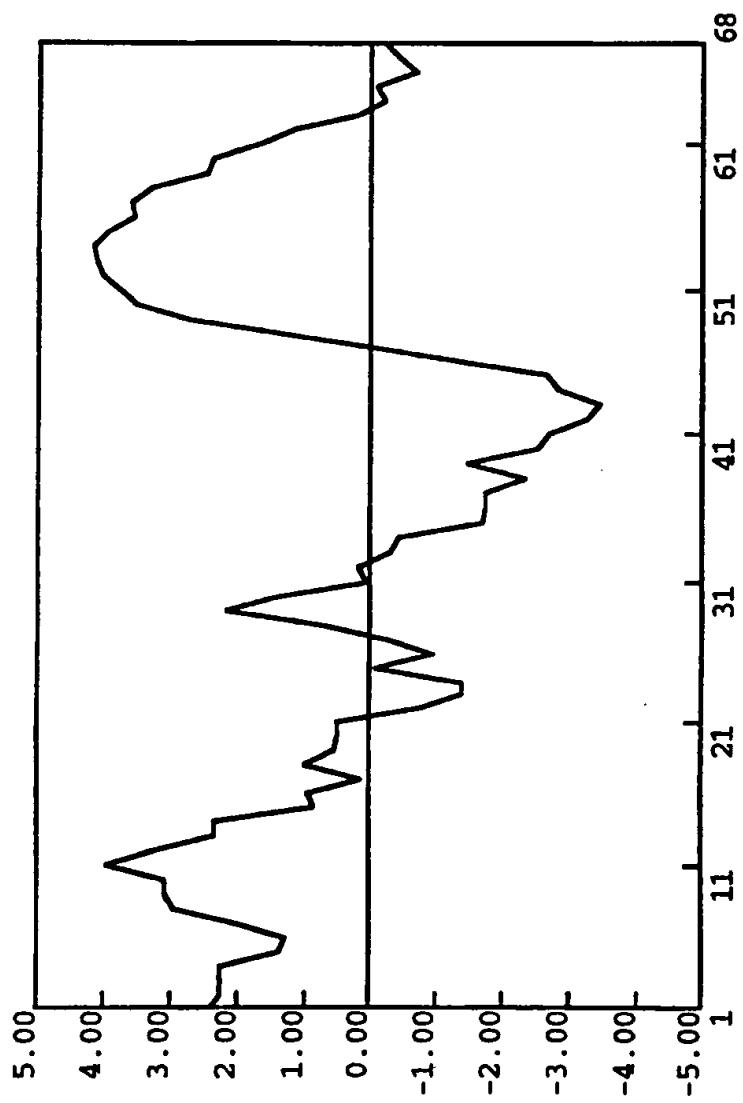
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FIGURE 58



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FIGURE 59



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FIGURE 60

p19P2 S12863	10	VWV[ELIV LV FAVRRH	20	SVTMLI[SH AL SDEV[ACTA	30	CVLL[LAFAF	40	50		
	1	LV[SH[AI	11	SVTMLI[SH SFSDL[AVM	12	CVLL[LAFAF	13	50		
p19P2 S12863	51	EPRGATVFC3G	60	LCH[VFEL[CP	70	VRYV[SVETL	80	TTIAYD[RVV	90	100
	51	MDH-[YFGET	52	MCK[PEVC	53	SVT[STESTL	54	VLIAVE[BQL	55	LVH[PLRRE[
p19P2 S12863	101	-	110	120	130	-	-	-	140	150
	101	NRHAYIGITV	111	TMVLA[VASSL	121	PFTVQQLTD	131	EPFQNVSLAA	141	FKDKYVCFDK
p19P2 S12863	151	----GLV	160	TyLPL[VLV	170	180	190	200	-	-
	151	FPSDSHRSY	161	TMVLDYF	171	GPLCFIFTY	181	VVRGCVTQSQ	191	NNMDKURDS
p19P2 S12863	201	ADWDRARR	210	TCMLWVWV	220	230	240	250	-	-
	201	KYRSSETKII	211	NWMLISIVA	221	VFA[EMLYY	231	241

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FIGURE 61

p19P2	1	10	VCAVCFVLLA	LVIARYVRLIH	IVINFLIGMI	ALSDVLMCTA	CVFELTLAYAF	50
PG3-2/pg1-10	1	10	ACIVGFLLLA	LVIARYVRLPL	IVINFLIGML	ALSDVLMCTA	CVFELTLAYAF	50
p19P2	51	60	EPFGAVFCGG	LECHLAVFLLCP	IVVYVSVFTL	ITIAVDRVV	IVHFLERRI-	100
PG3-2/pg1-10	51	60	EPFGAVFCGG	LECHLAVFLLCA	IVVYVSVFTL	ITIAVDRVV	IVVPLERRIS	100
p19P2	101	110	-----	-----	120	130	140	150
PG3-2/pg1-10	101	110	LRISAYAVLA	IWVLSAVTL	PAVHTHVE	-----	-----	150
p19P2	151	160	-----JILLY	IYLLPLLVIL	LSYVRVSVKI	RNRVVEGCCV	QSQAEIDRAF	200
PG3-2/pg1-10	151	160	QLYAWSLLLV	IYLLPLLVIL	LSYVRVSVKI	RNRVVEGRV	QSQAEIDRAF	200
p19P2	201	210	-----	-----	220	230	240	250
PG3-2/pg1-10	201	210	RRTFCCLVY	AVVFAICM	IVY	-----	-----	250

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FIGURE 62

9 18 27 36 45 54

5' CTC TGT GTC ATC GCG GTG GAT AGG TAC GTG GTT CTG GTG CAC CCG CTA CGT CGG
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Cys Val Ile Ala Val Asp Arg Tyr Val Val Leu Val His Pro Leu Arg Arg

63 72 81 90 99 108

CGC ATT TCA CTG AGG CTC AGC GCC TAC GCG GTG CTG GGC ATC TGG CCT CTA TCT
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Ile Ser Leu Arg Leu Ser Ala Tyr Ala Val Leu Gly Ile Trp Ala Leu Ser

117 126 135 144 153 162

GCA GTG CTG GCG CTG CGG CCC GCG GTG CAC ACC TAC CAT GTG GAG CTC AAG CCC
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Val Leu Ala Leu Pro Ala Ala Val His Thr Tyr His Val Glu Leu Lys Pro

171 180 189 198 207 216

CAC GAC GTG AGC CTC TGC GAG GAG TTC TGG GGC TCG CAG GAG CGC CAA CGC CAG
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
His Asp Val Ser Leu Cys Glu Glu Phe Trp Gly Ser Gln Glu Arg Gln Arg Gln

225 234 243 252 261 270

ATC TAC CCC TGG CGG CTG CTT CTG GGC ACC TAT TTG CTC CCC CTG CCC ATC
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Tyr Ala Trp Gly Leu Leu Gly Thr Tyr Leu Leu Pro Leu Leu Ala Ile

279 288 297 306 315 324

CTC CTG TCT TAC GTA CGG GTG TCA GTG AAG CTG AGG AAC CCC GTG GTG CCT GGC
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Leu Ser Tyr Val Arg Val Ser Val Lys Leu Arg Asn Arg Val Val Pro Gly

333 342 351 360 369 378

AGC GTG ACC CAG AGT CAA GCT GAC TGG GAC CGA GCG CGT CCC CGC CGC ACT TTC
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Val Thr Gln Ser Gln Ala Asp Trp Asp Arg Ala Arg Arg Arg Thr Phe

387 396 405 414 423 432

TGT CTG CTG GTG GTG GTA GTG TTC ACG CTC TGC TGG CTG CCC TTC TAC
 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Cys Leu Leu Val Val Val Val Phe Thr Leu Cys Trp Leu Pro Phe Tyr

CT 3'

--

—

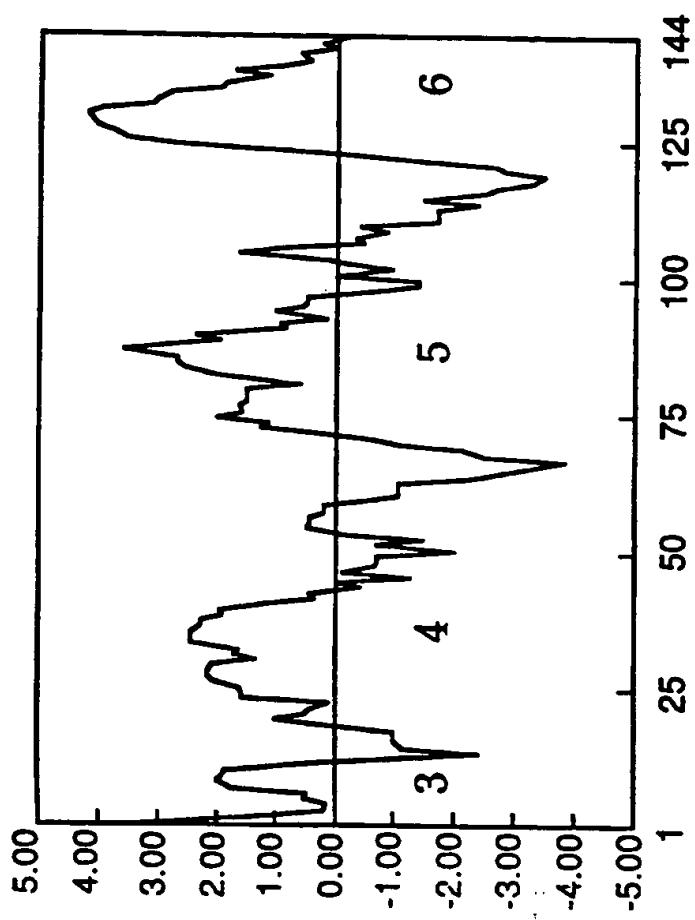
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FIGURE 63

p19P2	1	VGMVGNVY	10	LVVIARVRRLLH	20	MVINFLLIGENI	30	ALSDVLLNCTA	40	CVPPLTAYAF	50
PG3-2/pg1-10	1	VGMVGNMLLV	LVVIARVRRLLY			MVINFLLIGENI		ALSDVLLNCTA		CVPPLTAYAF	50
p5S38	-79	-30
p19P2	51	EFRGAVFGGC	60	LCHLIVFFLCP	70	VTIVVVSYFTI	80	ITIAVDRYVY	90	LVHPLURRRI	100
PG3-2/pg1-10	51	EFRGAVFGGC	51	ECHLIVFFLCA	51	VTIVVVSYFTI	51	ITIAVDRYVY	51	LVHPLURRRI	100
p5S38	-29	21
p19P2	101	URLSEAYAVIA	110	IPVLSAVLAI	120	PAAVHITYHVE	130	UKPHDVRLLCE	140	EFHGSQERQE	150
PG3-2/pg1-10	101	URLSEAYAVIA	101	IPVLSAVLAI	101	PAAVHITYHVE	101	UKPHDVRLLCE	101	EFHGSQERQE	150
p5S38	22	IPRLSEAYAVIG	IPVLSAVLAI	PAAVHITYHVE	UKPHDVRLLCE	EFHGSQERQE	71
p19P2	151	-----ELLLV	160	TYLLPLLVIL	170	LSYVRRVSVKI	180	EMRVVPGCVT	190	2SQADIDRAF	200
PG3-2/pg1-10	151	-----ELLLV	151	TYLLPLLVIL	151	LSYVRRVSVKI	151	EMRVVPGCVT	151	QSQADIDRAF	200
p5S38	72	-----ELLLV	72	TYLLPLLVIL	72	LSYVRRVSVKI	72	EMRVVPGCVT	72	QSQADIDRAF	121
p19P2	210	RRRTFCCLVA	220	VVVVF ₁ LCVL	230	FYF ₁	240	250	250
PG3-2/pg1-10	201	RRRTFCCLVA	201	VVVVF ₁ LCVL	201	FYF ₁	201	250	250
p5S38	122	RRRTFCCLVA	122	VVVVF ₁ LCVL	122	FYF ₁	122	171

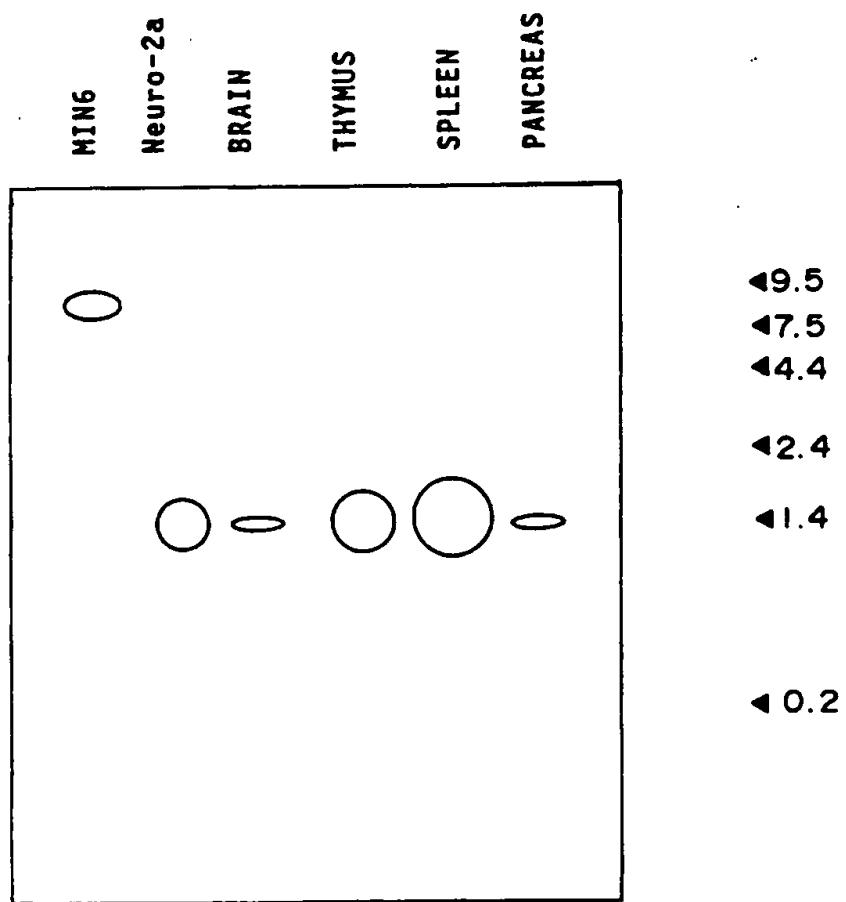
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FIGURE 64



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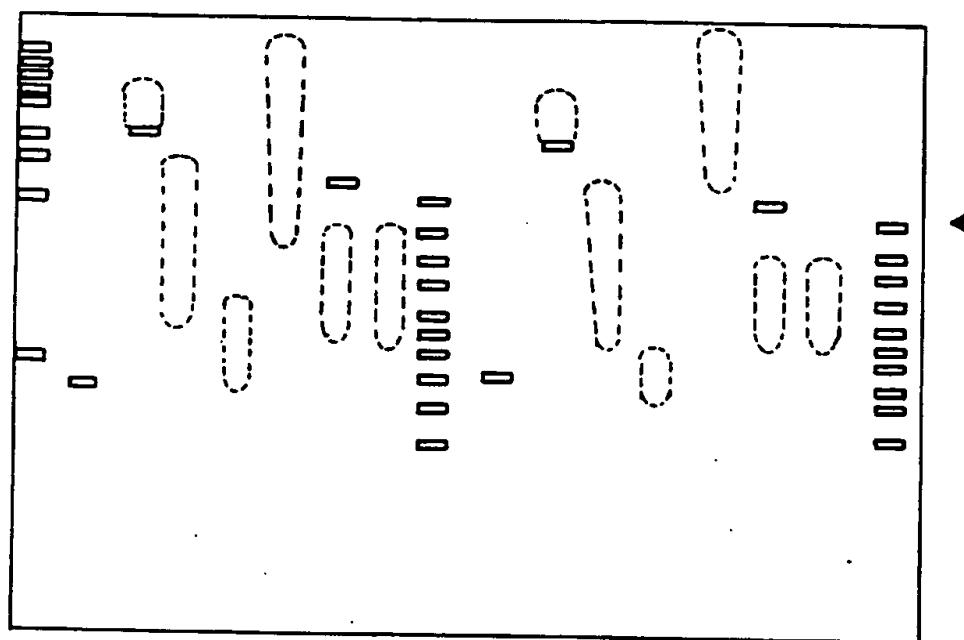
FIGURE 65



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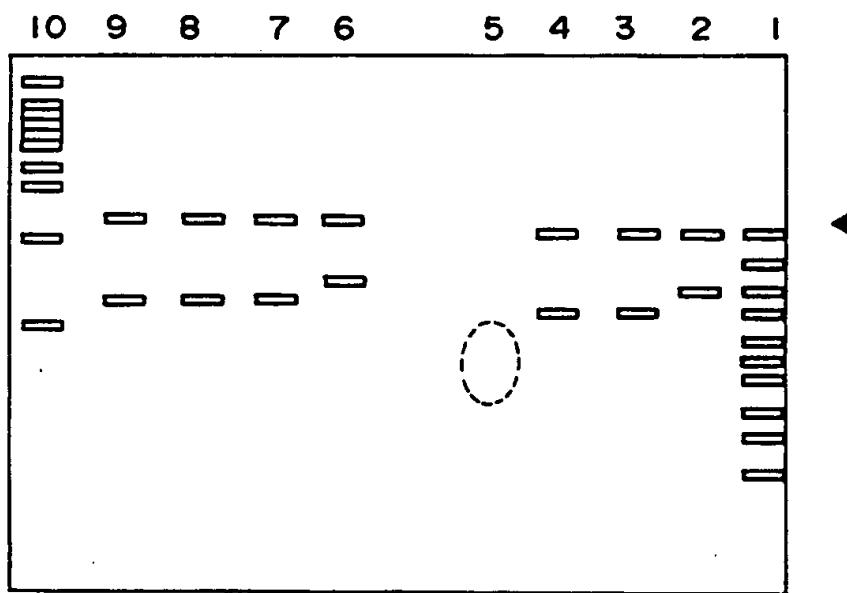
FIGURE 66

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17



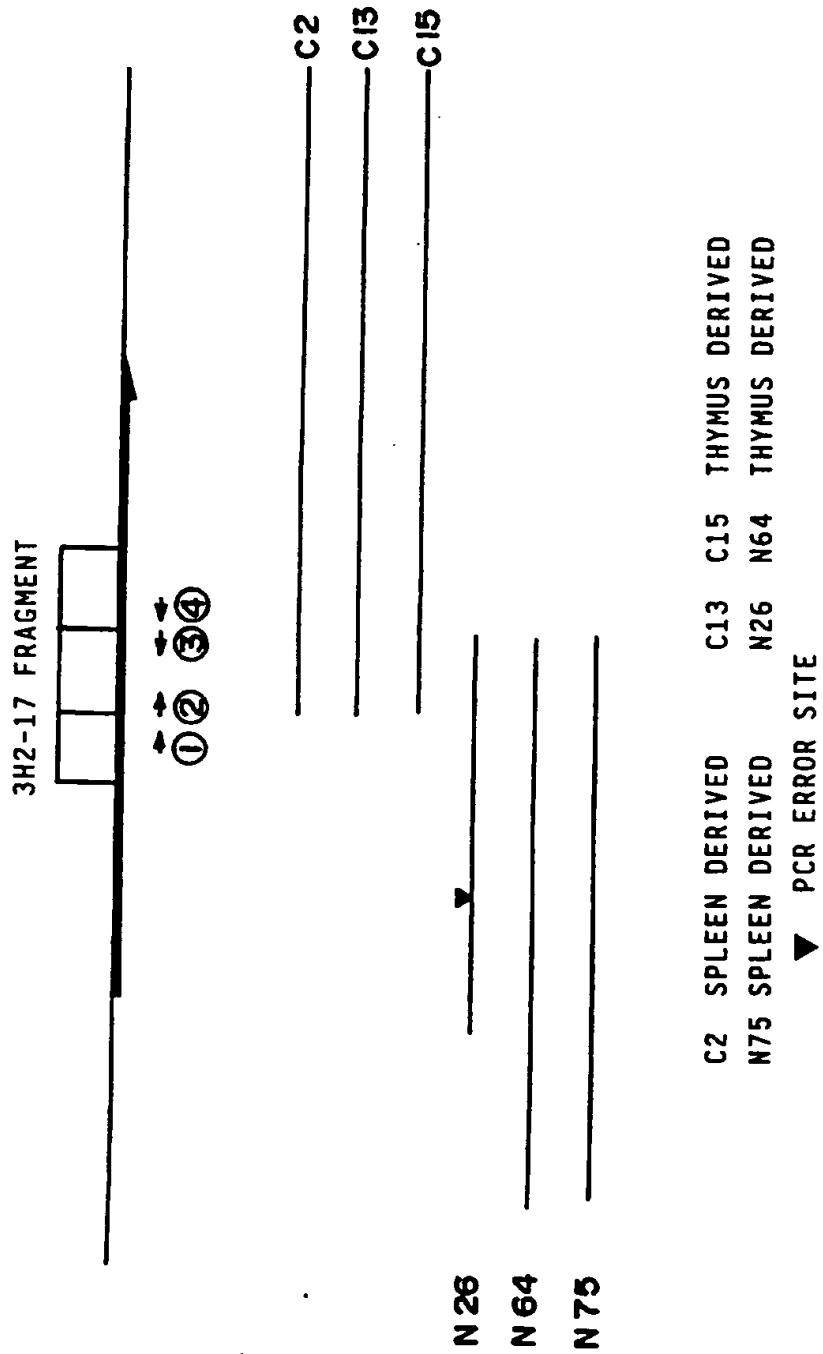
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FIGURE 67



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FIGURE 68



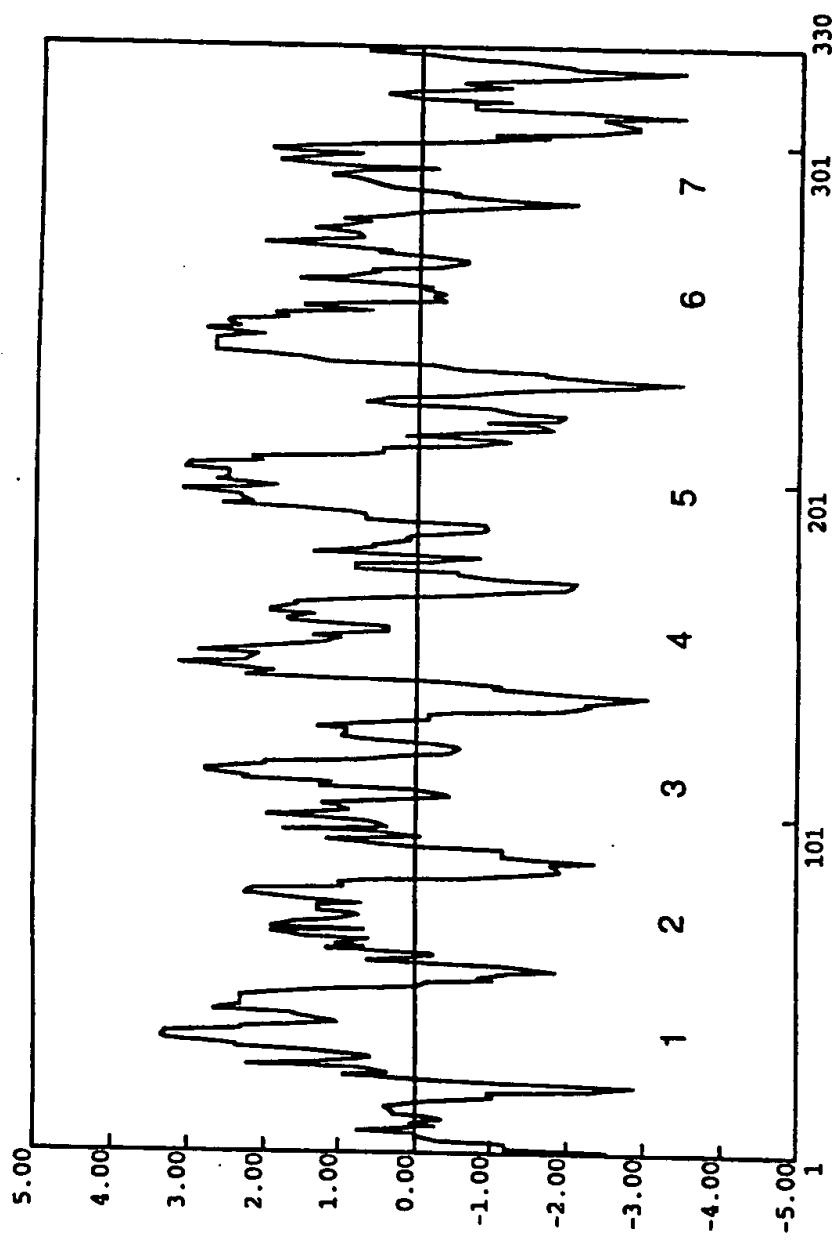
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FIGURE 69

1	GACCATAGGAAAGGCTGACAGCCAGTTATGGACCAGGACAAATGCCACCATCCAGGCTCCA 1	MetGluGlnAspAsnGlyThrIleGlnAlaPro	60 11
61	GCCTTGCGGCCACCACTGGCTTACCGTGAGGATTTCAGGCCACTGCTGCTAACCCCG 11	GlyLeuProProThrThrCysValTyrrArgGluAspPheLysArgLeuLeuLeuThrPro	120 31
121	GTATACTCGGTGGTGCTGGTGGTCGGCCTGCCACTGAACATCTGGCTCATGCCAGATC 31	ValTyrSerValValLeuValValGlyLeuProLeuAsnIleCysValIleAlaGlnIle	180 51
181	TGCCCATCCCCGGGACCTGACCCGTTCCCTGCTGACCCCTGAAACCTGGCACTGGCC 51	CysAlaSerArgArgThrLeuThrArgSerAlaValTyrThrLeuAsnLeuAlaLeuAla	240 71
241	GACCTGATGTATGCCCTGTTCACTACCCCTACTTATCTATAACTACGCCAGAGGGACCAC 71	AspLeuMetTyrAlaCysSerLeuProLeuLeuIleTyrAsnTyrAlaArgGlyAspHis	300 91
301	TGCCCTTCGGAGACCTGCCCTGCCGTTGTAACGCTTCCCTCTATGCCAATCTACAT 91	TrpProPheGlyAspLeuAlaCysArgPheValArgPheLeuPheTyrAlaAsnLeuHis	360 111
361	GGCAGCATCCCTGTTCTCACCTGCATTEAGCTTCCAGCGCTACCTGGCATCTGCCACCCC 111	GlySerIleLeuPheLeuThrCysIleSerPheGlnArgTyrLeuGlyIleCysHisPro	420 131
421	CTGGCTTCCCTGGCACAGCGTGGAGGTGGCGCTGCTGGTAGTGTGTGGAGTCGTC 131	LeuAlaSerTrpHisLysArgGlyArgArgAlaAlaTrpValValCysGlyValVal	480 151
481	TGGCTGGCTGIGACAGCCCAGTGCCTGCCAACGGCAGTCCTGGCTGCCACAGGCATCCAG 151	TrpLeuAlaValThrAlaGlnCysLeuProThrAlaValPheAlaAlaThrGlyIleGln	540 171
541	CGCAACCGCACTGTGCTTACGACCTGAGCCCACCCATCCTGCTACTCGCTACCTGCC 171	ArgAsnArgThrValCysTyrAspLeuSerProProIleLeuSerThrArgTyrLeuPro	600 191
601	TATGGTATGCCCTCACGGTCATGGCTTCTGCTGCCCTCATAGCCTTACTGGCTTGT 191	TyrGlyMetAlaLeuThrValIleGlyPheLeuLeuProPheIleAlaLeuLeuAlaCys	660 211
661	TATGGTGGCATGGCCGCCGCCCTGGTGCGGCCAGGATGGCCCAGCAGGTCTGTGGCCCAA 211	TyrCysArgMetAlaArgArgLeuCysArgGlnAspGlyProAlaGlyProValAlaGln	720 231
721	GACCGCGCACCAAGGCGGCTGTAAGGCTGGCTGGTGCGCAGCTGCTTGGCATCAGC 231	GluArgArgSerLysAlaAlaArgMetAlaValValAlaAlaValPheAlaIleSer	780 251
781	TTCTGGCTTTCACATCACCAAGACACCCCTACTTGGCTGTGGCTCCACGGGGGTGTC 251	PheLeuProPheHisIleThrIysThrAlaTyrLeuAlaValArgSerThrProGlyVal	840 271
841	TCTTGCCCTGCTGGAGACCTTCGCTGCTGCCAACAGGCACTGGCCCTGGCAGT 271	SerCysProValLeuGluThrPheAlaAlaAlaTyrLysGlyIhxArgProPheAlaSer	900 291
901	GTCAACAGTGTCTGGACCCATTCTCTACTTACACACAACAGAAAGTCCGGGGCMA 291	ValAsnSerValLeuAspProIleLeuPheTyrPheThrGlnGlnLysPheArgArgGln	960 311
961	CCCCACGATCTCTACAGAGGCTCACAGCCAGTOGCCAGAGCCAGAGACTCTGAGGCCCC 311	ProHisAspLeuLeuGlnArgLeuThrAlaIlysTrpGlnArgGlnArgVal***	1020 329

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FIGURE 70



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FIGURE 71

75+13, CODING	10	20	30	40	50	
P2UR_MOUSE	1	EDP-----	REHIAAC	PP-----	WYR-----	DPRL-----
P2YR_CHICK	1	AAEDEPANS	TIGGWEODE	GY-----	-----KCRFN-----	-----DERRV-----
	1	STEALISAAL	-----	LAGGWAAGN	A-----ESLXTKT	GDFYY-----
75+13, CODING	60	70	80	90	100	
P2UR_MOUSE	51	-----	-----	-----	-----	-----
P2YR_CHICK	51	-----	-----	-----	-----	-----
	51	-----	-----	-----	-----	-----
75+13, CODING	110	120	130	140	150	
P2UR_MOUSE	101	CSEPLLEIIN	AEI-----	LA-----	-----	-----
P2YR_CHICK	101	ASPLLLVIVY	EEI-----	-----RLK-----	VA-----	-----
	101	LT-----	FIC-----	M-----VPHMMP	EGI-----	-----
75+13, CODING	160	170	180	190	200	
P2UR_MOUSE	151	-----	-----	-----	-----	-----
P2YR_CHICK	151	-----	-----	-----	-----	-----
	151	-----	-----	-----	-----	-----
75+13, CODING	210	220	230	240	250	
P2UR_MOUSE	201	-----	-----	-----	-----	-----
P2YR_CHICK	201	-----	-----	-----	-----	-----
	201	-----	-----	-----	-----	-----
75+13, CODING	260	270	280	290	300	
P2UR_MOUSE	251	CRO-----	VIAQER-----	-----	-----	-----
P2YR_CHICK	251	-----	-----	-----	-----	-----
	251	-----	-----	-----	-----	-----
75+13, CODING	310	320	330	340	350	
P2UR_MOUSE	301	TP-----	PV-----	-----	-----	-----
P2YR_CHICK	301	-----	-----	-----	-----	-----
	301	-----	-----	-----	-----	-----
75+13, CODING	360	370	380	390	400	
P2UR_MOUSE	351	-----	-----	-----	-----	-----
P2YR_CHICK	351	-----	-----	-----	-----	-----
	351	-----	-----	-----	-----	-----
75+13, CODING	410	420	430	440	450	
P2UR_MOUSE	401	-----	-----	-----	-----	-----
P2YR_CHICK	401	-----	-----	-----	-----	-----
	401	-----	-----	-----	-----	-----

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FIGURE 72

9 18 27 36 45 54
 5' GCC ACC AAC GTG TTC ATC CTG TCA CTG GCC GAT GTG CTG GTG ACA GCC ATC TGC
 --- --- --- --- --- ---
 Ala Asp Val Leu Val Thr Ala Ile Cys

63 72 81 90 99 108
 CTG CCG GCC AGT CTG CTG GTA GAC ATC ACG GAA TCC TGG CTC TTT GCC CAT GCC
 --- --- --- --- --- ---
 Leu Pro Ala Ser Leu Leu Val Asp Ile Thr Glu Ser Trp Leu Phe Gly His Ala

117 126 135 144 153 162
 CTC TGC AAG GTC ATC CCC TAT CTA CAG GCC GTG TCC GTG TCA GTG GTC GTG CTG
 --- --- --- --- --- ---
 Leu Cys Lys Val Ile Pro Tyr Leu Gln Ala Val Ser Val Ser Val Val Leu

171 180 189 198 207 216
 ACT CTC AGC TCC ATC GCC CTG GAC CGC TGG TAC GCC ATC TGC CAC CCG CTG TTG
 --- --- --- --- --- ---
 Thr Leu Ser Ser Ile Ala Leu Asp Arg Trp Tyr Ala Ile Cys His Pro Leu Leu

225 234 243 252 261 270
 TTC AAG AGC ACT GCC CGG CGC GCC CGC GGC TCC ATC CTC GGC ATC TGG GCG GTG
 --- --- --- --- --- ---
 Phe Lys Ser Thr Ala Arg Arg Ala Arg Gly Ser Ile Leu Gly Ile Trp Ala Val

279 288 297 306 315 324
 TCG CTG GCT GTC ATG GTG CCT CAG GCT GCT GTC ATG GAG TGT AGC AGC GTG CTG
 --- --- --- --- --- ---
 Ser Leu Ala Val Met Val Pro Gln Ala Ala Val Met Glu Cys Ser Ser Val Leu

333 342 351 360 369 378
 CCC GAG CTG GCC AAC CGC ACC CGC CTC CTG TCT GTC TGT GAT GAG CGC TGG GCA
 --- --- --- --- --- ---
 Pro Glu Leu Ala Asn Arg Thr Arg Leu Leu Ser Val Cys Asp Glu Arg Trp Ala

387 396 405 414 423 432
 GAC GAC CTG TAC CCC AAG ATC TAC CAC AGC TGC TTC ATT GTC ACC TAC CTG
 --- --- --- --- --- ---
 Asp Asp Leu Tyr Pro Lys Ile Tyr His Ser Cys Phe Phe Ile Val Thr Tyr Leu

441 450 459 468 477 486
 GCC CCA CTG GGC CTC ATG GCC ATG GCC TAT TTC CAG ATC TTC CGC AAG CTC TGG
 --- --- --- --- --- ---
 Ala Pro Leu Gly Leu Met Ala Met Ala Tyr Phe Gln Ile Phe Arg Lys Leu Trp

495 504 513 522 531 540
 GGC CGC CAG ATC CCC GGC ACC ACC TCG GCC CTG GTG CGC AAC TGG AAG CGG CCC

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FIGURE 73

Gly Arg Gln Ile Pro Gly Thr Thr Ser Ala Leu Val Arg Asn Trp Lys Arg Pro
549 558 567 576 585 594
TCA GAC CAG CTG GAC GAC CAG GGC CAG GGC CTG AGC TCA GAG CCC CAG CCC CGG

Ser Asp Gln Leu Asp Asp Gln Gly Gln Gly Leu Ser Ser Glu Pro Gln Pro Arg
603 612 621 630 639 648
GCC CGC GCC TTC CTG GCC GAG GTG AAA CAG ATG CGA GCC CGG AGG AAG ACG GCC

Ala Arg Ala Phe Leu Ala Glu Val Lys Gln Met Arg Ala Arg Arg Lys Thr Ala
657 666 675 684 693 702
AAG ATG CTG ATG GTG GTG CTG CTG GTC TTC GCC CTC TGC TAC CTG CCC ATC AGT

Lys Met Leu Met Val Val Leu Val Phe Ala Leu Cys Tyr Leu Pro Ile Ser
711 720 729 738 747 756
GTC CTC AAC GTC CTC AAG AGG GTC TTC GGG ATG TTC CGC CAA GCC AGC GAC CGA

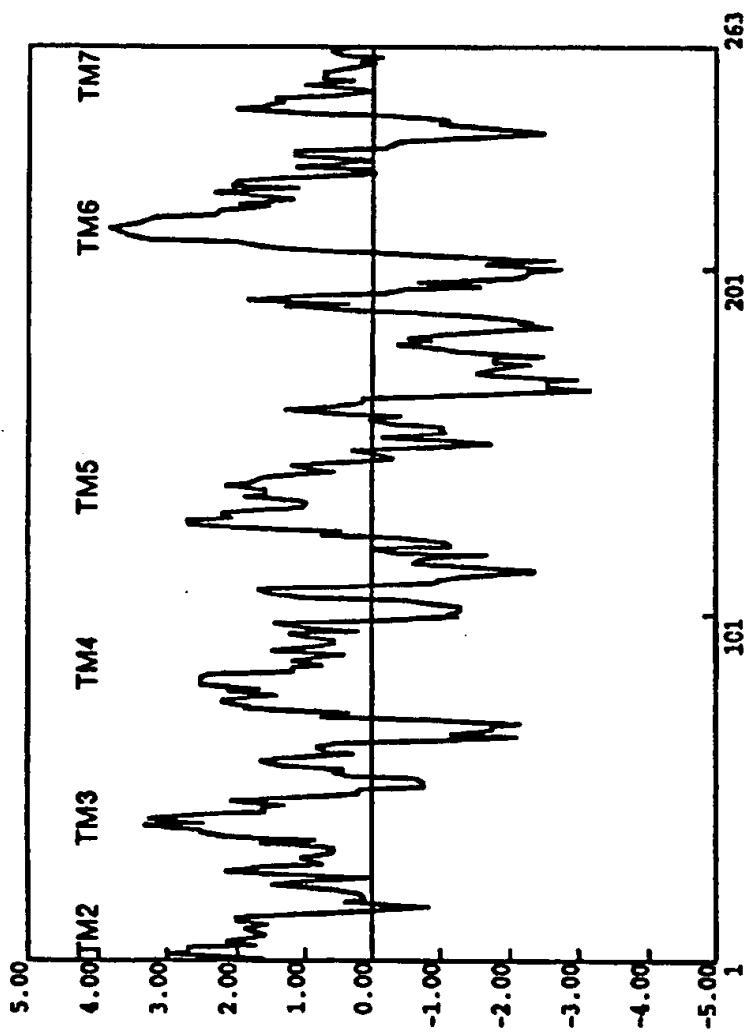
Val Leu Asn Val Leu Lys Arg Val Phe Gly Met Phe Arg Gln Ala Ser Asp Arg
765 774 783 792 801 810
GAG GCC ATC TAC GCC TGC TTC ACC TTC TCC CAC TGG CTG GTG TAC GCC AAC AGC

Glu Ala Ile Tyr Ala Cys Phe Thr Phe Ser His Trp Leu Val Tyr Ala Asn Ser
819 828 837
GCC GCC AAT CCC CTC CTC TAC TCC TTC CTC CCT 3'

Ala Ala

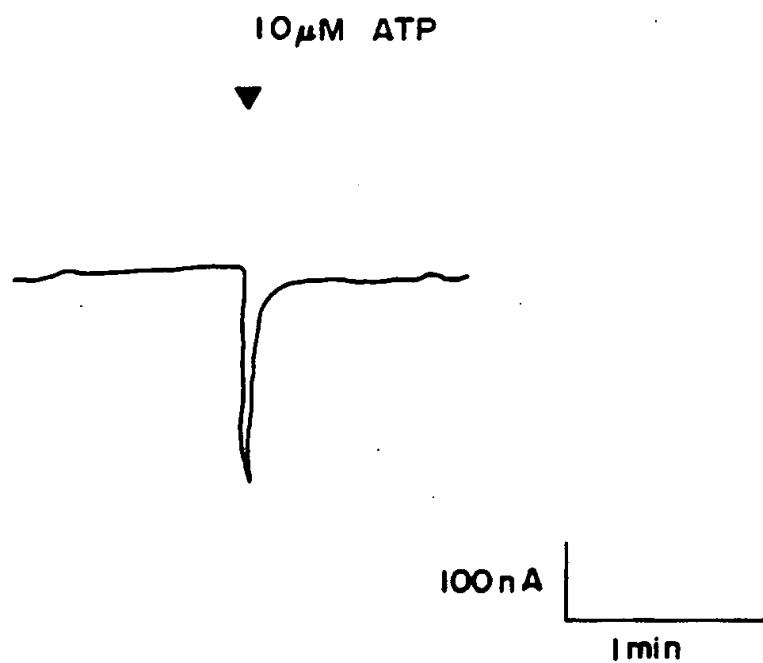
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FIGURE 74



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FIGURE 75



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FIGURE 76

		25	20	15	10	5	0	-5	-10	-15	-20	-25
h3H2-17(5'-)	1	GTCCTCTGG	TCCCAACAT	CCGGCTTCC	TGCCAAGC	GTGAGGTGC						
p3H2-17(5')	1											
h3H2-17(5'-)	51	CGCTCTGCT	TCCGAGTGT	GTGGAGTGT	GTGGCTGGT	GTGGCTGGT						
p3H2-17(5')	51											
h3H2-17(5'-)	101	GGCTTGCG	GGCTTGCG	GGCTTGCG	GGCTTGCG	GGCTTGCG						
p3H2-17(5')	101											
h3H2-17(5'-)	151	ATGCTGCT	ATGCTGCT	ATGCTGCT	ATGCTGCT	ATGCTGCT						
p3H2-17(5')	151											
h3H2-17(5'-)	201	GGCTTGCG	GGCTTGCG	GGCTTGCG	GGCTTGCG	GGCTTGCG						
p3H2-17(5')	201											
h3H2-17(5'-)	251	CTCTTCTT	CTCTTCTT	CTCTTCTT	CTCTTCTT	CTCTTCTT						
p3H2-17(5')	251											
h3H2-17(5'-)	301	CCAGCAGTC	CTGTCGCCA	AGAGCGGCC	AGCAAGGCC	CTCTTATGCC						
p3H2-17(5')	301											
h3H2-17(5'-)	351						
p3H2-17(5')	351						

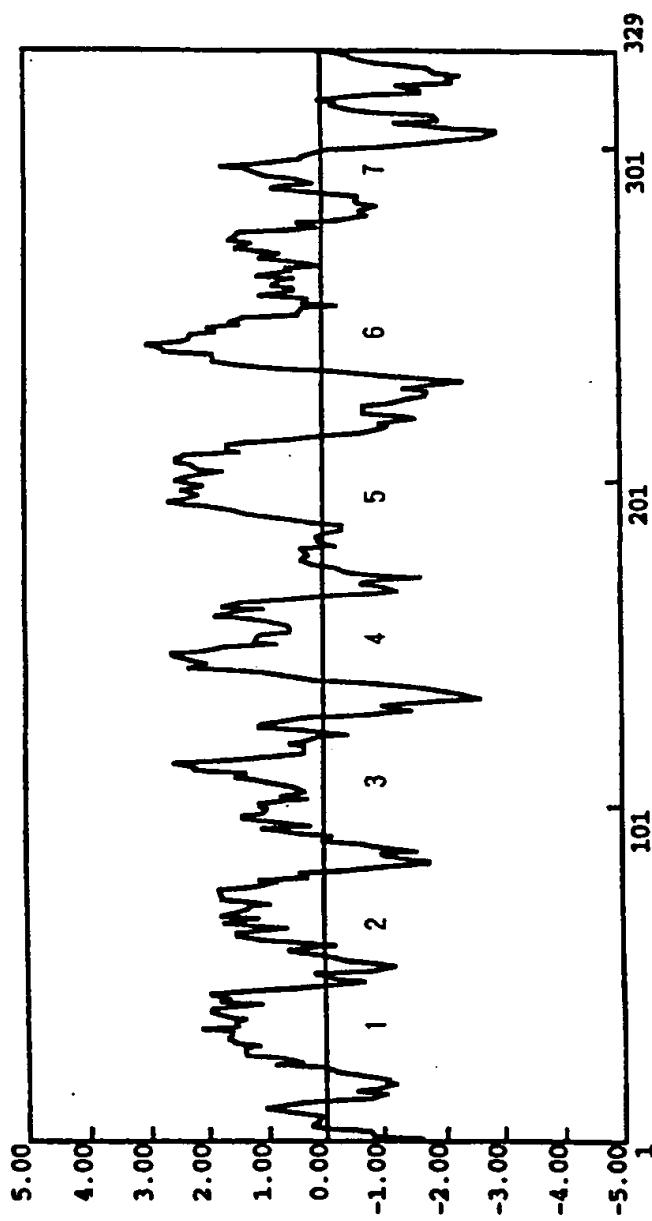
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FIGURE 77

1	TGACTCCCTGAACATAGGAAACCCACCTGGGCAGCCATGGAATGGGACAATGCCACAGGC MetGluTrpAspAsnGlyThrGly	60 8
61	CAGGCTCTGGCTTGCCACCCACCACCTGTGTCTACCGGGAGAACTTCAAGCACTGCTG 8 GlnAlaLeuGlyLeuProProThrThrCysValTyrArgGluAsnPheLysGlnLeuLeu	120 28
121	CTGCCACCTGTGTATTCCGGGTGCTGGGGCTGGCCTGCCGTCACATCTGTGTCATT 28 LeuProProValTyrSerAlaValLeuAlaAlaGlyLeuProLeuAsnIleCysValIle	180 48
181	ACCCAGATCTGCACGTCCCCGGGCGCTGACCCCCACGGGGCTGTACACCCCTAAACCTT 48 ThrGlnIleCysThrSerArgArgAlaLeuThrArgThrAlaValTyrThrLeuAsnLeu	240 68
241	GCTCTGGCTGACCTGCTATAATGCCCTGCCCTGCCCTGCTCATCACAACTATGCCAA 68 AlaLeuAlaAspLeuLeuTyrAlaCysSerLeuProLeuLeuIleTyrAsnTyrAlaGln	300 88
301	GGTGATCACTGGCCCTTGGCAGCTTGCCCTGCCGCTGGTCCGCTTCTCTCTATGCC 88 GlyAspHisTrpProPheGlyAspPheAlaCysArgLeuValArgPheLeuPheTyrAla	360 108
361	AACCTGCACGGCAGCATCTCTTCTCACCTGCATCAGCTTCCAGCGCTACCTGGGCATC 108 AsnLeuHisGlySerIleLeuPheLeuThrCysIleSerPheGlnArgLeuGlyIle	420 128
421	TGCCACCCGCTGGCCCCCTGGCACAAACGTTGGGGCCGGGGCTGCCCTGGCTAGTGTG 128 CysHisProLeuAlaProTrpHisLysArgGlyGlyArgArgAlaAlaTrpLeuValCys	480 148
481	GTAACCGTGTGGCTGGCCCTGACAACCCAGTGCCCTGCCAACAGCCATCTTCGCTGCCACA 148 ValThrValTrpLeuAlaValThrThrGlnCysLeuProThrAlaIlePheAlaAlaThr	540 168
541	GCCATCCAGCGTAACCGACTGCTGCTATGACCTCAGCCGCCCTGCCCTGGCCACCCAC 168 GlyIleGlnArgAsnArgThrValCysTyrAspLeuSerProProAlaLeuAlaThrHis	600 188
601	TATATGCCCTATGGCATGCCCTCACTGTCATGCCCTCCCTGCTGCCCTTGGCTGCCCTG 188 TyrMetProTyrGlyMetAlaLeuThrValIleGlyPheLeuLeuProPheAlaAlaLeu	660 208
661	CTGGCCTGCTACTGTCTCTGGCCCTGCCGCCCTGCCGCCAGGATGCCCGGAGAGCCT 208 LeuAlaCysTyrCysLeuLeuAlaCysArgLeuCysArgGlnAspGlyProAlaGluPro	720 228
721	GTGGCCCGAGGGCGGGCTGGCAAGGGGGCCCATGGCCCTGGCTGGCTGGCTGGCTGGCT 228 ValAlaGlnGluArgArgGlyLysAlaAlaArgMetAlaValValAlaAlaAlaPhe	780 248
781	GCCATCAGCTCCCTGCCCTTACATCACCAAGACAGCCATCCCTGGCAGTGGCTCGGACG 248 AlaIleSerPheLeuProPheHisIleThrLysThrAlaTyrLeuAlaValGlySerThr	840 268
841	CCGGGGCTCCCCCTGCACTGTATTGGAGGCCTTGCAGGGCCTACAAAGCCACGGGGCC 268 ProGlyValProCysThrValLeuGluAlaPheAlaAlaAlaTyrIysGlyThrArgPro	900 288
901	TTGCCAGTGCCAACAGCGTGCTGGACCCCATCCTCTCTACTTCACCCAGAAGAGTTC 288 PheAlaSerAlaAsnSerValLeuAspProIleLeuPheTyrPheThrGlnLysPhe	960 308
961	CCCCGGCGACCACATGAGCTCTACAGAAACTCACAGCCAAATGGCAGAGGCAGGGCTGG 308 ArgArgArgProHisGluLeuLeuGlnLysLeuThrAlaAlaLysTrpGlnArgGlnGlyArg	1020 328
1021	TGA 328 ***	1023 329

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FIGURE 78



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FIGURE 79

human prlno,	1	IEENGCTG	10	LGIPPTTCVY	20	RENFQLLP	30	EVYSVLTW	40	LPINICVIR	50
mouseFULL3H2	1	IEENGCTG	10	PGLPPPTTCVY	20	REDEKRLLLT	30	EVYSVLTW	40	LPINICVIR	50
human prlno,	51	ICCFERALTF	60	TAVYTLNIAL	70	ADLYACSLF	80	ELIINYAADC	90	AEPEGCCACE	100
mouseFULL3H2	51	ICCSRPLTTF	60	SAVYTLNIAL	70	ADLYACSLF	80	ELIINYAADC	90	AEPEGCCACE	100
human prlno,	101	LTEFLFYAM	110	AGSILFLTCI	120	EFCRYLGICH	130	PLASPHKRGCC	140	AEPAVTC	150
mouseFULL3H2	101	FTEFLFYANL	110	HGSILFLTCI	120	EFCRYLGICH	130	PLASPHKRGCC	140	AEPAVTC	150
human prlno,	151	PTLAVVIFCL	160	PTAIFATGII	170	PRTRIVCDL	180	SPETALHIV	190	PYCMLALVIC	200
mouseFULL3H2	151	PTLAVVIFCL	160	PTAIFATGII	170	PRTRIVCDL	180	SPETALHIV	190	PYCMLALVIC	200
human prlno,	201	PLLEFHITKL	210	PGCILPFIC	220	PGEGPESPV	230	JEPESEAKR	240	AVVVAEAFAT	250
mouseFULL3H2	201	PLLEFHITKL	210	PGCILPFIC	220	PGEGPESPV	230	JEPESEAKR	240	AVVVAEAFAT	250
human prlno,	251	FLPFHITKTI	260	AYLAVFSTEC	270	EPYIIEEP	280	AVYAGTRPFI	290	AVSVLDPII	300
mouseFULL3H2	251	FLPFHITKTI	260	AYLAVFSTEC	270	EPYIIEEP	280	AVYAGTRPFI	290	AVSVLDPII	300
human prlno,	301	FYFTKKEFRE	310	RPHELLGKT	320	SPENPFR*	330	AAVKGTRPFI	340	AVSVLDPII	350
mouseFULL3H2	301	FYFTKKEFRE	310	RPHELLGKT	320	SPENPFR*	330	AAVKGTRPFI	340	AVSVLDPII	350

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/JP 95/01599

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOLECULAR ENDOCRINOLOGY, vol. 5, January 1991 pages 1331-1338, M.T.HARRIGAN ET AL. 'Identification of a gene induced by glucocorticoids in murine T-cells : a potential G protein-coupled receptor' see the whole document ---	4-6, 10, 11, 13
Y	WO,A,92 01810 (LERNER MICHAEL R ;LERNER ETHAN A (US)) 6 February 1992 see abstract; claims 1-17 ---	14-18
Y	EP,A,0 578 962 (AMERICAN CYANAMID CO) 19 January 1994 see example 2 ---	14-18
A	----- -/-	1-3

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Patent family members are listed in annex.

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Date of the actual completion of the international search

18 December 1995

Date of mailing of the international search report

05.01.96

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INTERNATIONAL SEARCH REPORT

Internat'l Application No
PCT/JP 95/01599

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCIENCE, vol. 244, 1989 pages 569-571, LIBERT F. ET AL. 'Selective amplification and cloning of four new members of the G protein-coupled receptor family' cited in the application see the whole document -----	1-3

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

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		JP-A-	6153954	03-06-94
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